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51791

Date: 27 Sept. 01 Requester's Full Name: Examiner #: S. DEVI
Art Unit: 1645 Phone (308) 9347 Serial Number: 09/189,415
Results Format Preferred (circle): PAPER DISK E-MAIL

ensure an efficient and quality search, please attach a copy of the cover sheet, claims, and abstract or fill out the following:

Title of Invention: → BRETT B. FINLAY; BRENDANT KENNY;
Inventors (please provide full names): REBEKAH DE VINNEY; MARCUS STEIN.

Earliest Priority Date: 11.12.97

Search Topic:

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the cited species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known.

or Sequence Searches Only Please include all pertinent information (parent, grandchild, divisional, or issued patent numbers) along with appropriate serial number.

Please ask MS. BEVERLY SHEARS to perform this search.

Please see attached claims with key words highlighted and/or Examples and synonyms provided.

Please include the following databases: Embase, Medline, Biosis, CA (Dialog 50), JAPIO, JICTEplus, Dialog 35, 65, 77, 144, 256, 266, 440, 348, 357, 113, 129, 130, 156 and 60.

Please perform an inventor's name search.

Point of Contact:
Beverly Shears
Technical Info. Specialist
CM1 12C14 Tel: 308-4004

(STN)

SEP 27 2001

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Type of Search

Vendors and Cost

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Searcher Phone #: AA Sequence (#)
Searcher Location: Structure (#)
Searcher Picked Up: Bibliographic
Searcher Completed: 09-28-01 Litigation
Searcher Prep & Review Time: Fulltext
Searcher Time: Other

STN
Questel/Orbit
Lexis/Nexis
WWW/Internet
In-house sequence systems (list)
Other (specify)

Dev, S.
09/189415

09/189415

FILE 'REGISTRY' ENTERED AT 11:47:39 ON 28 SEP 2001

- key terms

E TRANSLOCATED INTIMIN RECEPTOR/CN

L3 3 SEA ABB=ON PLU=ON TRANSLOCATED INTIMIN RECEPTOR ?/CN

L11 18 S INTIMIN ?/CN

FILE 'CAPLUS' ENTERED AT 14:19:36 ON 28 SEP 2001

L1 (2348) SEA FILE=CAPLUS ABB=ON PLU=ON 90KD? OR 90(W) (KD? OR
KILOD? OR KILO(W) (D OR DA OR DALTON))

L2 (1011) SEA FILE=CAPLUS ABB=ON PLU=ON L1(5A) PROTEIN

L3 (3) SEA FILE=REGISTRY ABB=ON PLU=ON TRANSLOCATED INTIMIN
RECEPTOR ?/CN

L4 1609 SEA FILE=CAPLUS ABB=ON PLU=ON L2 OR L3 OR (TRANSLOCAT?
OR TRANS LOCAT?) (W) INTIMIN(W) RECEPTOR OR TIR OR HP90 OR
HP 90

L5 1209 SEA FILE=CAPLUS ABB=ON PLU=ON L4 AND (PROTEIN OR
POLYPEPTIDE OR POLYPROTEIN OR PEPTIDE)

L6 70 SEA FILE=CAPLUS ABB=ON PLU=ON L5 AND (EPEC OR EHEC OR
"A/E" OR ATTACH? (1W) EFFAC? OR (ENTEROPATHOGEN? OR
ENTEROHEMORRH? OR ENTEROHAEMORRH? OR ENTERO(W) (PATHOGEN?
OR HEMORRH? OR HAEMORRH?)) (5A) COLI)

L11 18 SEA FILE=REGISTRY ABB=ON PLU=ON INTIMIN ?/CN

L12 57 SEA FILE=CAPLUS ABB=ON PLU=ON L6 AND (L11 OR INTIMIN)

L13 34 SEA FILE=CAPLUS ABB=ON PLU=ON L12 AND BIND?

L13 ANSWER 1 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:699619 CAPLUS

TITLE: **Enteropathogenic E. coli**
tir binds Nck to initiate

actin pedestal formation in host cells
AUTHOR(S): Gruenheid, Samantha; DeViney, Rebekah; Bladt,
Friedhelm; Goosney, Danika; Gelkop, Sigal; Gish,
Gerald D.; Pawson, Tony; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, University of British
Columbia, Vancouver, BC, V6T 1G3, Can.

SOURCE: Nat. Cell Biol. (2001), 3(9), 856-859

CODEN: NCBIFN; ISSN: 1465-7392

PUBLISHER: Nature Publishing Group

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enteropathogenic Escherichia coli (EPEC**

) is a bacterial pathogen that causes infantile diarrhea worldwide.

EPEC injects a bacterial protein,

translocated intimin receptor (

Tir), into the host-cell plasma membrane where it acts as a
receptor for the bacterial outer membrane protein,

intimin. The interaction of Tir and

intimin triggers a marked rearrangement of the host actin

Searcher : Shears 308-4994

cytoskeleton into pedestals beneath adherent bacteria. On delivery into host cells, **EPEC Tir** is phosphorylated on tyrosine 474 of the intracellular carboxy-terminal domain, an event that is required for pedestal formation. Despite its essential role, the function of **Tir** tyrosine phosphorylation has not yet been elucidated. Here we show that tyrosine 474 of **Tir** directly **binds** the host-cell adaptor **protein** Nck, and that Nck is required for the recruitment of both neural Wiskott-Aldrich-syndrome **protein** (N-WASP) and the actin-related **protein** (Arp)2/3 complex to the **EPEC** pedestal, directly linking **Tir** to the cytoskeleton. Cells with null alleles of both mammalian Nck genes are resistant to the effects of **EPEC** on the actin cytoskeleton. These results implicate Nck adaptors as host-cell determinants of **EPEC** virulence.

L13 ANSWER 2 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:642816 CAPLUS

TITLE: **Intimin-specific immune responses prevent bacterial colonization by the attaching-effacing pathogen Citrobacter rodentium**

AUTHOR(S): Ghaem-Maghami, Marjan; Simmons, Cameron P.; Daniell, Sarah; Pizza, Mariagrazia; Lewis, David; Frankel, Gad; Dougan, Gordon

CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK

SOURCE: Infect. Immun. (2001), 69(9), 5597-5605

CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The formation of **attaching and effacing (A/E)** lesions on gut enterocytes is central to the pathogenesis of **enterohemorrhagic (EHEC) Escherichia coli**, **enteropathogenic E. coli (EPEC)**, and the rodent pathogen **Citrobacter rodentium**. Genes encoding **A/E** lesion formation map to a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Here we show that the LEE-encoded **proteins** **EspA**, **EspB**, **Tir**, and **intimin** are the targets of long-lived humoral immune responses in **C. rodentium**-infected mice. Mice infected with **C. rodentium** developed robust acquired immunity and were resistant to reinfection with wild-type **C. rodentium** or a **C. rodentium** deriv., DBS255(pCVD438), which expressed **intimin** derived from **EPEC** strain

E2348/69. The receptor-binding domain of **intimin polypeptides** is located within the carboxy-terminal 280 amino acids (Int280). Mucosal and systemic vaccination regimens using enterotoxin-based adjuvants were employed to elicit immune responses to recombinant Int280.alpha. from **EPEC** strain E2348/69. Mice vaccinated s.c. with Int280.alpha., in the absence of adjuvant, were significantly more resistant to oral challenge with DBS255(pCVD438) but not with wild-type *C. rodentium*. This type-specific immunity could not be overcome by employing an exposed, highly conserved domain of **intimin** (Int388-667) as a vaccine. These results show that anti-**intimin** immune responses can modulate the outcome of a *C. rodentium* infection and support the use of **intimin** as a component of a type-specific **EPEC** or **EHEC** vaccine.

REFERENCE COUNT: 45

REFERENCE(S): (1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
 (2) An, H; Microb Pathog 2000, V28, P291 CAPLUS
 (5) Batchelor, M; J Clin Microbiol 1999, V37, P3822 CAPLUS
 (7) Bouvet, J; Infect Immun 1999, V67, P2687 CAPLUS
 (8) China, B; Res Microbiol 1999, V150, P323 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 3 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:341382 CAPLUS

DOCUMENT NUMBER: 135:42490

TITLE: **Intimin** from Shiga toxin-producing *Escherichia coli* and its isolated C-terminal domain exhibit different **binding** properties for **Tir** and a eukaryotic surface receptor

AUTHOR(S): Deibel, Christina; Dersch, Petra; Ebel, Frank

CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, Germany

SOURCE: Int. J. Med. Microbiol. (2001), 290(8), 683-691
 CODEN: IMEMFV; ISSN: 1438-4221

PUBLISHER: Urban & Fischer Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The outer membrane **protein intimin** plays a crucial role in the **attaching and effacing** process employed by different enteropathogens to colonize the epithelial surface of their hosts. In this study the authors have characterized the C-terminal **binding** domain of **intimin** from the Shiga toxin-producing *E. coli* strain

413/89-1, that belongs to the .beta.-subtype of **intimins**. The authors found that a fusion of this domain to the maltose-binding protein binds efficiently to both the **translocated intimin receptor (Tir)** and the surface of uninfected eukaryotic host cells. In contrast, no such **binding** was obsd. with the full-length **protein** localized on the bacterial surface. As the C-terminal domain of **intimin** and the full-length **protein** differ in their **binding** activity, the authors suggest that the **intimin-binding** domain might be controlled by the N-terminal portion of the mol. to prevent unproductive interactions with mols. in the lumen of the gut.

REFERENCE COUNT: 30
 REFERENCE(S): (1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
 (2) Celli, J; Cell Microbiol 2000, V2, P1 CAPLUS
 (3) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS
 (4) Dersch, P; EMBO J 1999, V18, P1199 CAPLUS
 (5) Devinney, R; Cell Mol Life Sci 1999, V55, P961 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 4 OF 34 CAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 2001:309935 CAPLUS
 DOCUMENT NUMBER: 135:91417
 TITLE: Recruitment of cytoskeletal and signaling proteins to enteropathogenic and enterohemorrhagic Escherichia coli pedestals
 AUTHOR(S): Goosney, Danika L.; DeVinney, Rebekah; Finlay, B. Brett
 CORPORATE SOURCE: Biotechnology Laboratory and Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
 SOURCE: Infect. Immun. (2001), 69(5), 3315-3322
 CODEN: INFIBR; ISSN: 0019-9567
 PUBLISHER: American Society for Microbiology
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB **Enteropathogenic Escherichia coli (EPEC)** is a human pathogen that attaches to intestinal epithelial cells and causes chronic watery diarrhea. A close relative, **enterohemorrhagic E. coli (EHEC)**, causes severe bloody diarrhea and hemolytic-uremic syndrome. Both pathogens insert a **protein, Tir**, into the host cell plasma membrane where it **binds intimin**, the outer membrane ligand of **EPEC** and **EHEC**. This

interaction triggers a cascade of signaling events within the host cell and ultimately leads to the formation of an actin-rich pedestal upon which the pathogen resides. Pedestal formation is crit. in mediating **EPEC**- and **EHEC**-induced diarrhea, yet very little is known about its compn. and organization. In **EPEC**, pedestal formation requires **Tir** tyrosine 474 phosphorylation. In **EHEC** **Tir** is not tyrosine phosphorylated, yet the pedestals appear similar. The compn. of the **EPEC** and **EHEC** pedestals was analyzed by examg. numerous cytoskeletal, signaling, and adapter **proteins**. Of the 25 **proteins** examd., only two, calpactin and CD44, were recruited to the site of bacterial attachment independently of **Tir**. Several others, including ezrin, talin, gelsolin, and tropomyosin, were recruited to the site of **EPEC** attachment independently of **Tir** tyrosine 474 phosphorylation but required **Tir** in the host membrane. The remaining **proteins** were recruited to the pedestal in a manner dependent on **Tir** tyrosine phosphorylation or were not recruited at all. Differences were also found between the **EPEC** and **EHEC** pedestals: the adapter **proteins** Grb2 and CrkII were recruited to the **EPEC** pedestal but were absent in the **EHEC** pedestal. These results demonstrate that although **EPEC** and **EHEC** recruit similar cytoskeletal **proteins**, there are also significant differences in pedestal compn.

REFERENCE COUNT: 44
 REFERENCE(S): (1) Adam, T; J Cell Biol 1995, V129, P367 CAPLUS
 (2) Ben-Ami, G; Infect Immun 1998, V66, P1755 CAPLUS
 (4) Burrridge, K; Nature 1984, V308, P744 CAPLUS
 (5) Cantarelli, V; Infect Immun 2000, V68, P382 CAPLUS
 (6) Carlier, M; J Biol Chem 2000, V275, P21946 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 5 OF 34 CAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 2001:296138 CAPLUS
 DOCUMENT NUMBER: 135:90741
 TITLE: Site-directed mutagenesis of intimin .alpha. modulates intimin-mediated tissue tropism and host specificity
 AUTHOR(S): Reece, Stephen; Simmons, Cameron P.; Fitzhenry, Robert J.; Matthews, Stephen; Phillips, Alan D.; Dougan, Gordon; Frankel, Gad
 CORPORATE SOURCE: Centre for Molecular Microbiology and Infection, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7

2AZ, UK
 SOURCE: Mol. Microbiol. (2001), 40(1), 86-98
 CODEN: MOMIEE; ISSN: 0950-382X
 PUBLISHER: Blackwell Science Ltd.
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The hallmark of enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli* adhesion to host cells is intimate attachment leading to the formation of distinctive "attaching and effacing" lesions. This event is mediated, in part, by binding of the bacterial adhesion mol. intimin to a second bacterial protein, Tir, delivered by a type III secretion system into the host cell plasma membrane. The receptor-binding activity of intimin is localized to the C-terminal 280 amino acids (Int280) and at least five distinct intimin types (.alpha., .beta., .gamma., .delta. and .epsilon.) have been identified thus far. In addn. to binding to Tir, intimin can also bind to a component encoded by the host. The consequence of latter intimin-binding activity may det. tissue tropism and host specificity. In this study the authors selected three amino acids in intimin, which are implicated in Tir binding, for site-directed mutagenesis. The authors used the yeast two-hybrid system and gel overlays to study intimin-Tir protein interaction. In addn., the biol. consequences of the mutagenesis was tested using a no. of infection models (cultured epithelial cells, human intestinal explants and a mouse model). The authors report that while an I237/897A substitution (positions numbered according to Int280.alpha./whole intimin .alpha.) in intimin .alpha. did not have any affect on its biol. activity, a T255/914A substitution attenuated intimin activity in vivo. In contrast, the mutation V252/911A affected tissue targeting in the human intestinal explant model and attenuated the biol. activity of intimin in the mouse model. This study provides the first clues of the mol. basis of how intimin mediates tissue tropism and host specificity.

REFERENCE COUNT: 62

REFERENCE(S): (1) Abe, A; Mol Microbiol 1999, V33, P1162
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 (2) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
 (3) An, H; FEMS Microbiol Lett 1997, V148, P239 CAPLUS
 (5) Batchelor, M; EMBO J 2000, V19, P2452 CAPLUS
 (6) de Grado, M; Cell Microbiol 1999, V1, P7 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 6 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:184271 CAPLUS

DOCUMENT NUMBER: 134:217892

TITLE: Complete genome sequence of enterohemorrhagic
Escherichia coli O157:H7 and genomic comparison
with a laboratory strain K-12AUTHOR(S): Hayashi, Tetsuya; Makino, Kozo; Ohnishi, Makoto;
Kurokawa, Ken; Ishii, Kazuo; Yokoyama, Katsushi;
Han, Chang-Gyun; Ohtsubo, Eiichi; Nakayama,
Keisuke; Murata, Takahiro; Tanaka, Masashi;
Tobe, Toru; Iida, Tetsuya; Takami, Hideto;
Honda, Takeshi; Sasakawa, Chihiro; Ogasawara,
Naotake; Yasunaga, Teruo; Kuhara, Satoru; Shiba,
Tadayoshi; Hattori, Masahira; Shinagawa, HideoCORPORATE SOURCE: Department of Microbiology, Miyazaki Medical
College, Miyazaki, 899-1692, JapanSOURCE: DNA Res. (2001), 8(1), 11-22
CODEN: DARSE8; ISSN: 1340-2838

PUBLISHER: Universal Academy Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Escherichia coli O157:H7 is a major food-borne infectious pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. The complete chromosome sequence of an O157:H7 strain isolated from the Sakai outbreak is reported, and the results compared with the genome of a benign lab. strain, K-12 MG1655. The chromosome is 5.5 Mb in size, 859 Kb larger than that of K-12. A 4.1-Mb sequence highly conserved between the two strains is identified, which may represent the fundamental backbone of the E. coli chromosome. The remaining 1.4-Mb sequence comprises of O157:H7-specific sequences, most of which are horizontally transferred foreign DNAs. The predominant roles of bacteriophages in the emergence of O157:H7 is evident by the presence of 24 prophages and prophage-like elements that occupy more than half of the O157:H7-specific sequences. The O157:H7 chromosome encodes 1632 proteins and 20 tRNAs that are not present in K-12. Among these, at least 131 proteins are assumed to have virulence-related functions. Genome-wide codon usage anal. suggested that the O157:H7-specific tRNAs are involved in the efficient expression of the strain-specific genes. A complete set of the genes specific to O157:H7 presented here sheds new insight into the pathogenicity and the physiol. of O157:H7, and will open a way to fully understand the mol. mechanisms underlying the O157:H7 infection.

REFERENCE COUNT: 62

REFERENCE(S): (1) Altschul, S; Nucleic Acids Res 1997, V25,
P3389 CAPLUS

- (2) Amor, K; Infect Immun 2000, V68, P1116
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- (5) Bergthorsson, U; Mol Biol Evol 1998, V15, P6
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- (6) Blattner, F; Science 1997, V277, P1453
CAPLUS
- (9) Casjens, S; Mol Microbiol 2000, V35, P490
CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 7 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:98372 CAPLUS

DOCUMENT NUMBER: 134:232542

TITLE: Genome sequence of enterohemorrhagic Escherichia coli O157:H7

AUTHOR(S): Perna, Nicole T.; Plunkett, Guy, III; Burland, Valerie; Mau, Bob; Glasner, Jeremy D.; Rose, Debra J.; Mayhew, George F.; Evans, Peter S.; Gregor, Jason; Kirkpatrick, Heather A.; Posfai, Gyorgy; Hackett, Jeremiah; Klink, Sara; Boutin, Adam; Shao, Ying; Miller, Leslie; Grotbeck, Erik J.; Davis, N. Wayne; Lim, Alex; Dimalanta, Eileen T.; Potamouisis, Konstantinos D.; Apodaca, Jennifer; Anantharaman, Thomas S.; Lin, Jieyi; Yen, Glaex; Schwartz, David C.; Welch, Rodney A.; Blattner, Frederick R.

CORPORATE SOURCE: Genome Center of Wisconsin, Department of Animal Health and Biomedical Sciences, Laboratory of Genetics, Department of Chemistry, Department of Biostatistics, and Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, 53706, USA

SOURCE: Nature (London) (2001), 409(6819), 529-533
CODEN: NATUAS; ISSN: 0028-0836

PUBLISHER: Nature Publishing Group

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The bacterium Escherichia coli O157:H7 is a worldwide threat to public health and has been implicated in many outbreaks of hemorrhagic colitis, some of which included fatalities caused by hemolytic uremic syndrome. Close to 75,000 cases of O157:H7 infection are now estd. to occur annually in the United States. The severity of disease, the lack of effective treatment and the potential for large-scale outbreaks from contaminated food supplies have propelled intensive research on the pathogenesis and detection of E. coli O157:H7. The genome of E. coli O157:H7 was sequenced to identify candidate genes responsible for pathogenesis, to develop better methods of strain detection and to advance our understanding

of the evolution of *E. coli*, through comparison with the genome of the non-pathogenic lab. strain *E. coli* K-12. Lateral gene transfer found to be far more extensive than previously anticipated. In fact, 1387 new genes encoded in strain-specific clusters of diverse sizes were found in O157:H7. These include candidate virulence factors, alternative metabolic capacities, several prophages, and other new functions - all of which could be targets for surveillance.

REFERENCE COUNT: 30
 REFERENCE(S): (1) Alm, R; J Mol Med 1999, V77, P834 CAPLUS
 (2) Altschul, S; J Mol Biol 1990, V215, P403 CAPLUS
 (3) Blaisdell, J; J Bacteriol 1999, V181, P6396 CAPLUS
 (5) Blattner, F; Science 1997, V277, P1453 CAPLUS
 (6) Boyd, E; J Bacteriol 1997, V179, P1985 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 8 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:899327 CAPLUS
 DOCUMENT NUMBER: 134:204805
 TITLE: Interaction of the enteropathogenic
 Escherichia coli protein,
 translocated intimin
 receptor (tir), with focal
 adhesion proteins
 AUTHOR(S): Freeman, Nancy L.; Zurawski, Daniel V.;
 Chowrashi, Prokash; Ayooob, Joseph C.; Huang,
 Lily; Mittal, Balraj; Sanger, Jean M.; Sanger,
 Joseph W.
 CORPORATE SOURCE: Department of Cell and Developmental Biology,
 University of Pennsylvania School of Medicine,
 Philadelphia, PA, 19104-6058, USA
 SOURCE: Cell Motil. Cytoskeleton (2000), 47(4), 307-318
 CODEN: CMCYEO; ISSN: 0886-1544
 PUBLISHER: Wiley-Liss, Inc.
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB When enteropathogenic *Escherichia coli* (**EPEC**) attach and infect host cells, they induce a cytoskeletal rearrangement and the formation of cytoplasmic columns of actin filaments called pedestals. The attached **EPEC** and pedestals move over the surface of the host cell in an actin-dependent reaction [Sanger et al., 1996: Cell Motil Cytoskeleton 34:279-287]. The discovery that **EPEC** inserts the protein, translocated intimin

receptor (Tir), into the membrane of host cells, where it binds the EPEC outer membrane protein, intimin [Kenny et al., 1997: Cell 91:511-520], suggests Tir serves two functions: tethering the bacteria to the host cell and providing a direct connection to the host's cytoskeleton. The sequence of Tir predicts a protein of 56.8 kD with three domains sepd. by two predicted trans-membrane spanning regions. A GST-fusion protein of the N-terminal 233 amino acids of Tir (Tir1) binds to alpha-actinin, talin, and vinculin from cell exts. GST-Tir1 also coppts. purified forms of alpha-actinin, talin, and vinculin while GST alone does not bind these three focal adhesion proteins. Biotinylated probes of these three proteins also bound Tir1 cleaved from GST. Similar assocns. of alpha-actinin, talin, and vinculin were also detected with the C-terminus of Tir, i.e., Tir3, the last 217 amino acids. Antibody staining of EPEC-infected cultured cells reveals the presence of focal adhesion proteins beneath the attached bacteria. Our expts. support a model in which the cytoplasmic domains of Tir recruit a no. of focal adhesion proteins that can bind actin filaments to form pedestals. Since pedestals also contain villin, tropomyosin and myosin II [Sanger et al., 1996: Cell Motil. Cytoskeleton 34:279-287], the pedestals appear to be a novel structure sharing properties of both focal adhesions and microvilli.

REFERENCE COUNT:

31

REFERENCE(S):

- (1) Ayooob, J; Cell Motil Cytoskeleton 2000, V45, P67 CAPLUS
- (2) Bourdet-Sicard, R; EMBO J 1999, V18, P5853 CAPLUS
- (3) Chen, B; Mol Biol Cell 1999, V10, P4327 CAPLUS
- (4) Critchley, D; Biochem Soc Symp 1999, V65, P79 CAPLUS
- (5) Dabiri, G; Proc Natl Acad Sci USA 1990, V87, P6068 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 9 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2000:603715 CAPLUS

DOCUMENT NUMBER:

133:280269

TITLE:

Human response to Escherichia coli O157:H7 infection: antibodies to secreted virulence factors

AUTHOR(S):

Li, Yuling; Frey, Elizabeth; Mackenzie, Andrew M. R.; Finlay, B. Brett

CORPORATE SOURCE:

Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

09/189415

SOURCE: Infect. Immun. (2000), 68(9), 5090-5095
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vaccination has been proposed for the prevention of disease due to **enterohemorrhagic Escherichia coli (EHEC)**), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H7 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different **EHEC** virulence factors: **Tir** (**translocated intimin receptor**, which is inserted into the host cell membrane), **intimin** (bacterial outer membrane **protein** which **binds to Tir**), **EspA** (secreted **protein** which forms filamentous structures on **EHEC** surface), and **EspB** (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examd. Sera were assayed against purified recombinant **proteins** using immunoblot anal. and by ELISA to det. the sera's titers to each of the antigens in all patients. We found that there was little reaction to **EspA**, **EspB**, and **intimin** in the acute-phase sera, although there was some reactivity to **Tir**. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against **Tir** (up to a titer of 1:256,000), esp. in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for **Tir**. These results suggest that there is a strong immune response to **Tir**, and to a lesser extent to the other three virulence factors, following **EHEC** disease, indicating that these bacterial mols. are potential vaccine candidates for preventing **EHEC** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (**Tir** or **EspB**) are still recognized by the host immune response.

REFERENCE COUNT: 2
REFERENCE(S): (1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) Bitzan, M; J Clin Microbiol 1992, V30, P1174
MEDLINE

L13 ANSWER 10 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:535365 CAPLUS
DOCUMENT NUMBER: 133:155433
TITLE: Inhibitors of **intimin** adhesion and tests for their screening

Searcher : Shears 308-4994

09/189415

INVENTOR(S): Frankel, Gad Meir; Matthews, Stephen John; Hale,
Christine Betty; Dougan, Gordon
PATENT ASSIGNEE(S): Imperial College Innovations Limited, UK
SOURCE: PCT Int. Appl., 96 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000045173	A1	20000803	WO 2000-GB254	20000131
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: GB 1999-1897 A 19990129

AB The invention relates to the provision of **polypeptides** which comprise or consist of the **Tir binding** domain of **intimin** and/or a **Tir-independent** eukaryotic cell **binding** activity and to the use of such **polypeptides** in methods of screening for agents which affect the **binding** of **intimin** to an eukaryotic cell, preferably an intestinal cell. Such inhibitors are useful in the prevention or treatment of bacterial infections, esp. those which cause diarrhea.

IT 287121-95-3

RL: BOC (Biological occurrence); BPR (Biological process); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)

(amino acid sequence; inhibitors of **intimin** adhesion and tests for antidiarrheal screening)

REFERENCE COUNT: 15

REFERENCE(S): (2) Armstrong; US 5858698 A 1999 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

Searcher : Shears 308-4994

L13 ANSWER 11 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:519996 CAPLUS

DOCUMENT NUMBER: 133:249419

TITLE: Expression of **intimin** .gamma. from
enterohemorrhagic Escherichia coli in *Citrobacter rodentium*AUTHOR(S): Hartland, Elizabeth L.; Huter, Veronika;
Higgins, Lisa M.; Goncalves, Nathalie S.;
Dougan, Gordon; Phillips, Alan D.; MacDonald,
Thomas T.; Frankel, GadCORPORATE SOURCE: Department of Biochemistry, Imperial College of
Science, Technology and Medicine, London, SW7
2AZ, UKSOURCE: Infect. Immun. (2000), 68(8), 4637-4646
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The carboxy-terminal 280 amino acids (Int280) of the bacterial adhesion mol. **intimin** include the receptor-binding domain. At least 5 different types of Int280, designated .alpha., .beta., .gamma., .delta., and .epsilon., have been described based on sequence variation in this region. Importantly, the **intimin** types are assocd. with different evolutionary branches and contribute to distinct tissue tropism of **intimin**-pos. bacterial pathogens. This study describes how a strain of *C. rodentium*, which normally displays **intimin** .beta., was engineered to express **intimin** .gamma. from **enterohemorrhagic E. coli**. **Intimin** .gamma. bound to the **translocated intimin receptor (Tir)** from *C. rodentium* and had the ability to produce **attaching** and **effacing** lesions on HEP-2 cells. However, *C. rodentium* expressing **intimin** .gamma. could not colonize orally infected mice or induce mouse colonic hyperplasia. These results suggest that **intimin** may contribute to host specificity, possibly through its interaction with a receptor on the host cell surface.

REFERENCE COUNT: 33

REFERENCE(S): (1) Adu-Bobie, J; J Clin Microbiol 1998, V36,
P662 CAPLUS
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CAPLUS
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CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 12 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:497087 CAPLUS

DOCUMENT NUMBER: 133:219121

TITLE: Crystal structure of enteropathogenic
Eschenchia coli intimin
-receptor complex

AUTHOR(S): Luo, Yu; Frey, Elizabeth A.; Pfuetzner, Richard
A.; Creagh, A. Louise; Knoechel, Derek G.;
Haynes, Charles A.; Inlay, B. Brett; Strynadka,
Natalie C. J.

CORPORATE SOURCE: Department of Biochemistry and Molecular
Biology, University of British Columbia,
Vancouver, BC, V6T 1Z3, Can.

SOURCE: Nature (London) (2000), 405(6790), 1073-1077
CODEN: NATUAS; ISSN: 0028-0836

PUBLISHER: Nature Publishing Group

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Intimin and its translocated intimin
receptor (Tir) are bacterial proteins
that mediate adhesion between mammalian cells and attaching
and effacing (A/E) pathogens.
Enteropathogenic Escherichia coli (EPEC)
causes significant paediatric morbidity and mortality world-wide. A
related A/E pathogen, enterohaemorrhagic
E. coli (EHEC; 0157:H7) is one of the most
important food-borne pathogens in North America, Europe and Japan.
A unique and essential feature of A/E bacterial
pathogens is the formation of actin-rich pedestals beneath the
intimately adherent bacteria and localized destruction of the
intestinal brush border. The bacterial outer membrane adhesin,
intimin, is necessary for the prodn. of the A/
E lesion and diarrhea. The A/E bacteria
translocate their own receptor for intimin, Tir,
into the membrane of mammalian cells using the type III secretion
system. The translocated Tir triggers addnl. host
signaling events and actin nucleation, which are essential for
lesion formation. Here we describe the crystal structures of an
EPEC intimin carboxyterminal fragment alone and in
complex with the EPEC Tir intimin-
binding domain, giving insight into the mol. mechanisms of
adhesion of A/E pathogens.

REFERENCE COUNT: 30

REFERENCE(S): (3) Banner, D; J Mol Biol 1987, V196, P657
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CAPLUS
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V276, P472 CAPLUS
- ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 13 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:472429 CAPLUS

DOCUMENT NUMBER: 133:219083

TITLE: Structural basis for recognition of the
translocated intimin
receptor (Tir) by

intimin from enteropathogenic
Escherichia coli

AUTHOR(S): Batchelor, Miranda; Prasannan, Sunil; Daniell,
Sarah; Reece, Stephen; Connerton, Ian;
Bloomberg, Graham; Dougan, Gordon; Frankel, Gad;
Matthews, Stephen

CORPORATE SOURCE: Department of Biochemistry, Imperial College of
Science, Technology and Medicine, London, SW7
2AZ, UK

SOURCE: EMBO J. (2000), 19(11), 2452-2464

CODEN: EMJODG; ISSN: 0261-4189

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Intimin** is a bacterial adhesion mol. involved in intimate attachment of **enteropathogenic** and **enterohaemorrhagic Escherichia coli** to mammalian host cells. **Intimin** targets the **translocated intimin receptor (Tir)**, which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the **Tir-binding** region of **intimin** to the C-terminal 190 amino acids (Int190). We have also detd. the region's high-resoln. soln. structure, which comprises an Ig domain that is intimately coupled to a novel C-type lectin domain. This fragment, which is necessary and sufficient for **Tir** interaction, defines a new super domain in **intimin** that exhibits striking structural similarity to the **integrin-binding** domain of the *Yersinia* invasin and C-type lectin families. The extracellular portion of **intimin** comprises an articulated rod of Ig domains extending from the bacterium surface, conveying a highly accessible "adhesive tip" to the target cell. The interpretation of

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NMR-titrn. and mutagenesis data has enabled us to identify, for the first time, the **binding** site for **Tir**, which is located at the extremity of the Int190 moiety.

REFERENCE COUNT: 59

REFERENCE(S): (1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
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L13 ANSWER 14 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:438489 CAPLUS

DOCUMENT NUMBER: 133:161648

TITLE: Mechanical fractionation reveals structural requirements for **enteropathogenic** *Escherichia coli* **Tir** insertion into host membranes

AUTHOR(S): Gauthier, Annick; De Grado, Myriam; Finlay, B. Brett

CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Infect. Immun. (2000), 68(7), 4344-4348

CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enteropathogenic** *Escherichia coli* (**EPEC**

) inserts its receptor for intimate adherence (**Tir**) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial **protein** delivery into mammalian cells. In this study, the Triton X-100-sol. membrane fraction from **EPEC**-infected HeLa cells was contaminated with bacterial **proteins**. Therefore, a mech. method of cell lysis and ultracentrifugation to fractionate infected HeLa cells was applied to investigate the biol. and biochem. of **Tir** delivery and translocation. This method demonstrates that the translocation of **Tir** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or **binding** to **Tir**'s ligand, **intimin**.

REFERENCE COUNT: 31

REFERENCE(S): (1) Abe, A; Mol Microbiol 1999, V33, P1162 CAPLUS

Searcher : Shears 308-4994

- (2) Bauer, M; Trends Cell Biol 2000, V10, P25
CAPLUS
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P961 CAPLUS
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CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 15 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:431539 CAPLUS

DOCUMENT NUMBER: 133:174359

TITLE: **Intimin from enteropathogenic**
Escherichia coli mediates remodelling
of the eukaryotic cell surface

AUTHOR(S): Phillips, Alan D.; Giron, Jorge; Hicks, Susan;
Dougan, Gordon; Frankel, Gad

CORPORATE SOURCE: University Department of Paediatric
Gastroenterology, Royal Free Hospital, London,
NW3 2QG, UK

SOURCE: Microbiology (Reading, U. K.) (2000), 146(6),
1333-1344

CODEN: MROBEO; ISSN: 1350-0872

PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Adhesion to cultured epithelial cells by **enteropathogenic**
Escherichia coli (EPEC) is assocd. with
extensive rearrangement of the host cell cytoskeleton. Evidence has
been presented that EPEC adhesion is assocd. with
activation of signal transduction pathways leading to prodn. of a
characteristic histopathol. feature known as the **attaching**
and **effacing (A/E)** lesion. A
/E lesion formation requires **intimin**, an
EPEC adhesion mol. and several EPEC secreted
proteins (EspA, B, D and Tir) involved in cell
signalling and **protein** translocation. In this study it is
shown that HEp-2 cells respond during the early stages of infection
with two wild-type EPEC strains (B171 and E2348/69) by
producing microvillus-like processes (MLP) at the site of initial
bacterial adherence. **Intimin** appears to play a key role
in MLP elongation. At later stages of infection with these
wild-type EPEC strains, when A/E
lesions have formed, the MLP were reduced in no. and length to
appear as at time zero, and the cell surface in the vicinity of
bacterial clusters appeared unaffected. In contrast, infection with
EspA- or EspB-neg., but **intimin**-pos., EPEC

strains (UMD872 and UMD864, resp.) resulted in enhanced MLP proliferation and formation of cage-like structures engulfing the bacteria. Inoculating HEp-2 cells with intimin-coated latex spheres induced similar cage-like structures. Caco-2 cells did not show intimin-induced microvillus elongation in response to EPEC infection, although microvillus effacement and redn. in no. occurred. Similar phenomena appeared on B171 and E2348/69 infection of pediatric intestine using in vitro organ culture, i.e. elongated microvilli were seen in assocn. with small colonies and at the periphery of large localized colonies, along with evidence of microvillus breakdown and debris in the colony center. These results show that intimin activates signal transduction pathways involved in the remodelling of the eukaryotic cell surface, probably via binding to a receptor encoded by the host cell.

REFERENCE COUNT:

44

REFERENCE(S):

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 16 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:421954 CAPLUS

DOCUMENT NUMBER: 133:147284

TITLE: Enteropathogenic E. coli
translocated intimin
receptor, Tir, interacts
directly with .alpha.-actinin

AUTHOR(S): Goosney, Danika L.; DeVinney, Rebekah;
Pfuetzner, Richard A.; Frey, Elizabeth A.;
Strynadka, Natalie C.; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, The Department of
Microbiology and Immunology, University of
British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Curr. Biol. (2000), 10(12), 735-738
CODEN: CUBLE2; ISSN: 0960-9822

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC
) triggers a dramatic rearrangement of the host epithelial cell

actin cytoskeleton to form an **attaching** and **effacing** lesion, or pedestal. The pathogen remains attached extracellularly to the host cell through the pedestal for the duration of the infection. At the tip of the pedestal is a bacterial **protein**, **Tir**, which is secreted from the bacterium into the host cell plasma membrane, where it functions as the receptor for an **EPEC** outer membrane **protein**, **intimin** [1]. Delivery of **Tir** to the host cell results in its tyrosine phosphorylation, followed by **Tir-intimin binding**. **Tir** is believed to anchor **EPEC** firmly to the host cell, although its direct linkage to the cytoskeleton is unknown. Here, we show that **Tir** directly **binds** the cytoskeletal **protein** .alpha.-actinin. .alpha.-Actinin is recruited to the pedestal in a **Tir**-dependent manner and colocalizes with **Tir** in infected host cells. **Binding** is mediated through the amino terminus of **Tir**. Recruitment of .alpha.-actinin occurs independently of **Tir** tyrosine phosphorylation. Recruitment of actin, VASP, and N-WASP, however, is abolished in the absence of this tyrosine phosphorylation. These results suggest that **Tir** plays at least three roles in the host cell during infection: **binding intimin** on **EPEC**; mediating a stable anchor with .alpha.-actinin through its amino terminus in a phosphotyrosine-independent manner; and recruiting addnl. cytoskeletal **proteins** at the carboxyl terminus in a phosphotyrosine-dependent manner. These findings demonstrate the first known direct linkage between extracellular **EPEC**, through the transmembrane **protein** **Tir**, to the host cell actin cytoskeleton via .alpha.-actinin.

REFERENCE COUNT:

14

REFERENCE(S):

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CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 17 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:298095 CAPLUS

DOCUMENT NUMBER: 133:71203

TITLE: Identification of the **intimin-binding** domain of **Tir** of

enteropathogenic Escherichia coli

AUTHOR(S): De Grado, Myriam; Abe, Akio; Gauthier, Annick; Steele-Mortimer, Olivia; DeVinney, Rebekah; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Cell. Microbiol. (1999), 1(1), 7-17
CODEN: CEMIF5; ISSN: 1462-5814

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC

) attaches intimately to mammalian cells via a bacterial outer membrane adhesion mol., **intimin**, and its receptor in the host cell membrane, **Tir**. **Tir** is a bacterial **protein** translocated into the host cell membrane and tyrosine phosphorylated after insertion. **Tir-intimin binding** induces organized actin polymn. beneath the adherent bacteria, resulting in the formation of pedestal-like structures. A series of **Tir** deletion derivs. were constructed to analyze which **Tir** domains are involved in **intimin binding**. We have localized the **intimin-binding** domain (IBD) of **Tir** using a yeast two-hybrid system and a gel-overlay approach to a region of 109 amino acids that is predicted to be exposed on the surface of the plasma membrane. A truncated **Tir protein** lacking this domain was translocated to the host cell membrane and tyrosine phosphorylated, but failed to **bind intimin** or to induce either actin polymn. or **Tir** accumulation beneath the bacteria. These results indicate that only a small region of **Tir** is needed to **bind intimin** and support the predicted topol. for **Tir**, with both N- and C-terminal regions in the mammalian cell cytosol. They also confirm that **Tir-intimin** interactions are needed for cytoskeletal organization. We have also identified N-terminal regions involved in **Tir** stability and **Tir** secretion to the media.

REFERENCE COUNT: 27

- REFERENCE(S):**
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L13 ANSWER 18 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:279346 CAPLUS

DOCUMENT NUMBER: 133:295077

TITLE: Human colostrum and serum contain antibodies reactive to the **intimin-binding** region of the **enteropathogenic Escherichia coli translocated intimin receptor**

AUTHOR(S): Sanches, Marcela Imperio; Keller, Rogeria; Hartland, Elizabeth L.; Figueiredo, Dayse M. M.; Batchelor, Miranda; Martinez, Marina B.; Dougan, Gordon; Careiro-Sampaio, Magda M. S.; Frankel, Gad; Trabulsi, Luiz R.

CORPORATE SOURCE: Departamento de Microbiologia, Instituto de Ciencias Biomedicas, Departamento de Immunologia, ICB III and Faculdade de Ciencias Farmaceutica, Departamento de Analises Clinicas e Toxicologicas Universidade de Sao Paulo, Sao Paulo, Brazil

SOURCE: J. Pediatr. Gastroenterol. Nutr. (2000), 30(1), 73-77

CODEN: JPGND6; ISSN: 0277-2116

PUBLISHER: Lippincott Williams & Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Background: In Brazil, **enteropathogenic Escherichia coli (EPEC)** diarrhoea is endemic in young infants. A characteristic feature of **EPEC** adhesion to host cells is intimate attachment leading to the formation of distinctive "**attaching and effacing**" (A/E) lesions on mammalian cells. Two genes directly involved in intimate adhesion, **eae** and **tir**, encode the adhesion mol. **intimin** and its translocated receptor **Tir**, resp. The **intimin-binding** domain of **Tir** was recently mapped to the middle part of the **polypeptide** (**Tir-M**), and the amino (**Tir-N**) and carboxy (**Tir-C**) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a no. of **proteins** assocd. with **EPEC** virulence. It has also been shown that patients infected with verocytotoxin-producing **E. coli** O157 can produce antibodies to **Tir**. In the current study antibody responses to the different **Tir** domains were analyzed in sera and colostrum samples collected in an

EPEC-endemic area of Brazil. Methods: Recombinant **Tir**, **Tir-N**, **Tir-M**, and **Tir-C** were expressed as His-tagged protein in *E. coli* BL21a and purified on nickel columns. Western blot anal. was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the **Tir** fragments. Results: Anti-**Tir** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea. Anti-**Tir** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the **Tir-polypeptide**, **Tir M**, was identified. Conclusion: The **intimin-binding** region of **Tir** (**Tir-M**) is the immunodominant region of the **polypeptide** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the **Tir-M** domain.

REFERENCE COUNT:

21

REFERENCE(S):

- (1) Adu-Bobie, J; J Clin Microbiol 1998, V36, P662 CAPLUS
- (3) Camara, L; Int Arch Allergy Immunol 1994, V103, P307 CAPLUS
- (4) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
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L13 ANSWER 19 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2000:51065 CAPLUS

DOCUMENT NUMBER:

133:16094

TITLE:

Antibody response of patients infected with verocytotoxin-producing *Escherichia coli* to protein antigens encoded on the LEE locus

AUTHOR(S):

Jenkins, C.; Chart, H.; Smith, H. R.; Hartland, E. L.; Batchelor, M.; Delahay, R. M.; Dougan, G.; Frankel, G.

CORPORATE SOURCE:

Laboratory of Enteric Pathogens, Central Public Health Laboratory, London, NW9 5HT, UK

SOURCE:

J. Med. Microbiol. (2000), 49(1), 97-101

CODEN: JMMIAV; ISSN: 0022-2615

PUBLISHER:

Lippincott Williams & Wilkins

DOCUMENT TYPE:

Journal

LANGUAGE:

English

AB Sera from patients infected with verocytotoxin-producing *Escherichia coli* (VTEC) O157, from patients with antibodies to *E. coli* O157

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lipopolysaccharide (LPS) and from healthy controls were examd. for antibodies to **proteins** involved in expressing the **attaching and effacing** phenotype. After SDS-PAGE, purified recombinant **intimin**, EspA-filament structural **protein**, translocated **protein** EspB and three sep. domains of the **translocated intimin receptor (Tir)** were tested for reaction with patients' sera by immunoblotting. An ELISA was also used to detect antibodies to **intimin** in sera from E. coli O157 LPS antibody-pos. individuals. Seven of nine culture-pos. patients and one control patient had antibodies to EspA. Five of these patients and two controls had serum antibodies to the **intimin-binding** region of **Tir**, whereas none of the sera contained antibodies **binding** to either of the intracellular domains of **Tir**. By immunoblotting, 10 of 14 culture-pos. patients had antibodies to the conserved region of **intimin**, eight of whom were infected with E. coli O157 phage type 2. Thirty six of 60 sera from culture-neg. but E. coli O157 LPS antibody-pos. patients had antibodies to **intimin** as detd. by ELISA. The secreted **proteins** are expressed in vivo during infection and are considered as pathogenic markers. Antibodies to these **proteins** may form the basis of a serodiagnostic test for the detection of patients infected with VTEC which carry the locus for the enterocyte effacement pathogenicity island and provide an adjunct test to the established serol. tests based on VTEC LPS.

REFERENCE COUNT: 16
REFERENCE(S): (1) Adu-Bobie, J; Infect Immun 1998, V66, P5643
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L13 ANSWER 20 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:26661 CAPLUS
DOCUMENT NUMBER: 132:176540
TITLE: Hierarchy in the expression of the locus of
enterocyte effacement genes of
enteropathogenic Escherichia coli
AUTHOR(S): Friedberg, Devorah; Umanski, Tatiana; Fang,
Yuan; Rosenshine, Ilan
CORPORATE SOURCE: Departments of Molecular Genetics and

Searcher : Shears 308-4994

09/189415

SOURCE: Biotechnology, Faculty of Medicine, The Hebrew University, Jerusalem, 91120, Israel
Mol. Microbiol. (1999), 34(5), 941-952
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Enteropathogenic Escherichia coli (EPEC)**
) elicit changes in host cell morphol. and cause actin rearrangement, a phenotype that has commonly been referred to as **attaching/effacing (AE)** lesions. The ability of **EPEC** to induce AE lesions is dependent upon a type III **protein** secretion/translocation system that is encoded by genes clustered in a 35.6 kb DNA segment, named the locus of enterocyte effacement (LEE). The authors used transcriptional fusions between the green fluorescent **protein (gfp)** reporter gene and LEE genes *rorf2*, *orf3*, *orf5*, *escJ*, *escV* and *eae*, together with immunoblot anal. with antibodies against **Tir**, **intimin**, *EspB* and *EspF*, to analyze the genetic regulation of the LEE. The expression of all these LEE genes was strictly dependent upon the presence of a functional integration host factor (IHF). IHF **binds** specifically upstream from the *ler* (*orf1*) promoter and appears to activate expression of *ler*, *orf3*, *orf5* and *rorf2* directly. The *ler*-encoded **Ler protein** was involved in activating the expression of *escJ*, *escV*, **tir**, *eae*, *espB* and *espF*. Expression of both IHF and **Ler** was needed to elicit actin rearrangement assocd. with AE lesions. In conclusion, IHF directly activates the expression of the *ler* and *rorf2* transcriptional units, and **Ler** in turn mediates the expression of the other LEE genes.

REFERENCE COUNT: 27
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(6) Elliott, S; Mol Microbiol 1998, V28, P1 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 21 OF 34 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:675935 CAPLUS
DOCUMENT NUMBER: 132:20883
TITLE: The **Tir-binding** region of **enterohemorrhagic Escherichia coli intimin** is sufficient to trigger actin condensation after

Searcher : Shears 308-4994

AUTHOR(S): bacterial-induced host cell signalling
 Liu, Hui; Magoun, Lorraine; Luperchio, Steve;
 Schauer, David B.; Leong, John M.
 CORPORATE SOURCE: Department of Molecular Genetics and
 Microbiology, University of Massachusetts
 Medical Center, Worcester, MA, 01655, USA
 SOURCE: Mol. Microbiol. (1999), 34(1), 67-81
 CODEN: MOMIEE; ISSN: 0950-382X
 PUBLISHER: Blackwell Science Ltd.
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB **Enterohemorrhagic Escherichia coli (EHEC)**
) has emerged as an important agent of diarrheal disease.
 Attachment to host cells, an essential step during intestinal
 colonization by **EHEC**, is assocd. with the formation of a
 highly organized cytoskeletal structure contg. filamentous actin,
 termed an **attaching and effacing (A/E)** lesion, directly beneath bound bacteria. The outer
 membrane **protein intimin** is required for the
 formation of this structure, as is **Tir**, a bacterial
protein that is translocated into the host cell and is
 thought to function as a receptor for **intimin**. To
 understand **intimin** function better, the authors fused
EHEC intimin to a homologous **protein**,
Yersinia pseudotuberculosis invasin, or to maltose-binding
protein. The N-terminal 539 amino acids of **intimin**
 were sufficient to promote outer membrane localization of the
 C-terminus of invasin and, conversely, the N-terminal 489 amino
 acids of invasin were sufficient to promote the localization of the
 C-terminus of **intimin**. The C-terminal 181 residues of
intimin were sufficient to bind mammalian cells
 that had been preinfected with an **enteropathogenic E.**
coli strain that expresses **Tir** but not
intimin. Binding of **intimin** derivs. to
 preinfected cells correlated with **binding** to recombinant
Tir protein. Finally, the 181-residue minimal
Tir-binding region of **intimin**, when
 purified and immobilized on latex beads, was sufficient to trigger
A/E lesions on preinfected mammalian cells.

REFERENCE COUNT: 60
 REFERENCE(S): (3) Deibel, C; Mol Microbiol 1998, V28, P463
 CAPLUS
 (4) Dersch, P; EMBO J 1999, V18, P1199 CAPLUS
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 CAPLUS
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CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 22 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:632014 CAPLUS

DOCUMENT NUMBER: 131:333663

TITLE: Identification of CestT, a chaperone for the type III secretion of **Tir** in **enteropathogenic Escherichia coli**

AUTHOR(S): Elliott, Simon J.; Hutcheson, Steven W.; Dubois, Maria S.; Mellies, Jay L.; Wainwright, Leslie A.; Batchelor, Miranda; Frankel, Gad; Knutton, Stuart; Kaper, James B.

CORPORATE SOURCE: Center for Vaccine Development and Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, 21201, USA

SOURCE: Mol. Microbiol. (1999), 33(6), 1176-1189
CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The locus of enterocyte effacement of **enteropathogenic Escherichia coli** encodes a type III secretion system, an outer membrane **protein** adhesin (**intimin**, the product of **eae**) and **Tir**, a translocated **protein** that becomes a host cell receptor for **intimin**. Many type III secreted **proteins** require chaperones, which function to stabilize **proteins**, prevent inappropriate **protein-protein** interactions and aid in secretion. An open reading frame located between **tir** and **eae**, previously named **orfU**, was predicted to encode a **protein** with partial similarity to the *Yersinia* **SycH** chaperone. The authors examd. the potential of the **orfU** gene product to serve as a chaperone for **Tir**. The **orfU** gene encoded a 15 kDa cytoplasmic **protein** that specifically interacted with **Tir** as demonstrated by the yeast two-hybrid assay, column binding and coimmunopptn. expts. An **orfU** mutant was defective in **attaching-effacing** lesion formation and **Tir** secretion, but was unaffected in expression of other virulence factors. **OrfU** appeared to stabilize **Tir** levels in the cytoplasm, but was not absolutely necessary for secretion of **Tir**. Based upon the phys. similarities, phenotypic characteristics and the demonstrated interaction with **Tir**, **orfU** is redesignated as **cestT** for the chaperone for *E. coli* secretion of **Tir**.

REFERENCE COUNT: 47

REFERENCE(S) : (1) Abe, A; Mol Microbiol 1999, V33 CAPLUS
 (2) Anderson, D; Science 1997, V278, P1140 CAPLUS
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 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 23 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:632013 CAPLUS

DOCUMENT NUMBER: 131:333662

TITLE: **Enteropathogenic Escherichia coli translocated intimin receptor, Tir**, requires a specific chaperone for stable secretion

AUTHOR(S) : Abe, Akio; De Grado, Myriam; Pfuetzner, Richard A.; Sanchez-SanMartin, Claudia; DeVinney, Rebekah; Puente, Jose Luis; Strynadka, Natalie C. J.; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Mol. Microbiol. (1999), 33(6), 1162-1175

CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Enteropathogenic Escherichia coli (EPEC)** secretes several Esps (E. coli-secreted **proteins**) that are required for full virulence. Insertion of the bacterial **protein Tir** into the host epithelial cell membrane is facilitated by a type III secretion app., and at least EspA and EspB are required for **Tir** translocation. An **EPEC** outer membrane **protein, intimin**, interacts with **Tir** on the host membrane to establish intimate attachment and formation of a pedestal-like structure. In this study, we identified a **Tir** chaperone, CestT, whose gene is located between **tir** and **eae** (which encodes **intimin**). A mutation in **cestT** abolished **Tir** secretion into culture supernatants and significantly decreased the amt. of **Tir** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp **proteins**. The level of **tir** mRNA was not affected by the **cestT** mutation, indicating that CestT acts at the post-transcriptional level. The **cestT** mutant could not induce host cytoskeletal rearrangements, and displayed the

same phenotype as the **tir** mutant. Gel overlay and GST pulldown assays demonstrated that CestT specifically interacts with **Tir**, but not with other Esp proteins. Furthermore, by using a series of **Tir** deletion derivs., we detd. that the CestT binding domain is located within the first 100 amino-terminal residues of **Tir**, and that the pool of **Tir** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for **Tir** secretion, and at least the first 200 residues of **Tir** were required for efficient secretion. Gel filtration studies showed that **Tir**-CestT forms a large multimeric complex. Collectively, these results indicate that CestT is a **Tir** chaperone that may act as an anti-degrdn. factor by specifically binding to its amino-terminus, forming a multimeric stabilized complex.

REFERENCE COUNT: 57
 REFERENCE(S): (1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
 (2) An, H; FEMS Microbiol Lett 1997, V148, P239 CAPLUS
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 (4) Beaudry, M; J Clin Microbiol 1996, V34, P144 CAPLUS
 (6) Cheng, L; Mol Microbiol 1997, V24, P757 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 24 OF 34 CAPLUS COPYRIGHT 2001 ACS
 ACCESSION NUMBER: 1999:569268 CAPLUS
 DOCUMENT NUMBER: 131:307586
 TITLE: A novel chromosomal locus of enteropathogenic Escherichia coli (EPEC), which encodes a bfpT-regulated chaperone-like protein, trcA, involved in microcolony formation by EPEC
 AUTHOR(S): Tobe, Toru; Tatsuno, Ichiro; Katayama, Eisaku; Wu, Cheng-Yen; Schoolnik, Gary K.; Sasakawa, Chihiro
 CORPORATE SOURCE: Department of Bacteriology, University of Tokyo, Tokyo, 108-0071, Japan
 SOURCE: Mol. Microbiol. (1999), 33(4), 741-752
 CODEN: MOMIEE; ISSN: 0950-382X
 PUBLISHER: Blackwell Science Ltd.
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB The bfpTVW operon, also known as the per operon, of enteropathogenic Escherichia coli (EPEC)

is required for the transcriptional activation of the bfp operon, which encodes the major subunit and assembly machinery of bundle-forming pili (BFP). An immobilized T7-tagged BfpT fusion protein that binds specifically to upstream promoter sequences of bfpA and eae was used to "fish out" from a promoter library other EPEC chromosomal fragments that are bound by the BfpT protein. After screening for promoters exhibiting bfpTVW-dependent expression, one was identified that was pos. regulated by bfpTVW and that is not present in the chromosomes of two non-virulent E. coli lab. strains, DH5.alpha. and HB101. Further anal. of this pos. regulated promoter in EPEC showed that it resided within a 4.9 kb sequence that is not present in E. coli K12. This locus, located downstream of the potB gene, was found to contain four open reading frames (ORFs): bfpTVW-activated promoter was localized upstream of ORF1. An ORF1 knockout mutant produced less of the BFP structural subunit (BfpA) and formed smaller than normal adherent microcolonies on cultured epithelial cells; however, this mutation did not affect bfp transcription. An ORF1-His6 fusion protein specifically bound the preprocessed and mature forms of the BfpA protein and thus appears to stabilize the former within the cytoplasmic compartment. ORF1 therefore is a newly isolated EPEC chromosomal gene that encodes a chaperone-like protein involved in the prodn. of BFP. Hence, ORF1 was designated trcA (bfpT-regulated chaperone-like protein gene). The TrcA protein also specifically bound 39 kDa and 90 kDa proteins that are expressed by EPEC but not by E. coli K12. The 90 kDa protein was revealed to be intimin, a protein product of the eae gene, which is required for the EPEC attaching/effacing phenotype, suggesting a direct interaction of TrcA with intimin in the cytoplasmic compartment.

REFERENCE COUNT:

46

REFERENCE(S):

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CAPLUS
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CAPLUS
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 - (6) Brosius, J; Gene 1984, V27, P151 CAPLUS
- ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 25 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:326051 CAPLUS

DOCUMENT NUMBER: 130:333761

TITLE: Pathogenic Escherichia coli intimin
receptor Tir and gene tir

09/189415

and methods for detecting gene **tir** or
Tir protein and for drug
screening
INVENTOR(S): Finlay, B. Brett; Kenny, Brendan; Devinney,
Rebekah; Stein, Marcus
PATENT ASSIGNEE(S): University of British Columbia, Can.
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9924576	A1	19990520	WO 1998-CA1042	19981110
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9911373	A1	19990531	AU 1999-11373	19981110
EP 1029054	A1	20000823	EP 1998-954076	19981110
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
PRIORITY APPLN. INFO.:			US 1997-65130	P 19971112
			WO 1998-CA1042	W 19981110

AB A polypeptide, called **Tir** (for translocated intimin receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *E. coli* is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic *E. coli* can be performed by the use of antibodies which bind to **Tir** to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding **Tir polypeptide**. Isolated nucleic acid sequences encoding **Tir polypeptide**, **Tir peptides**, a recombinant method for producing recombinant **Tir**, antibodies which bind to **Tir**, and a kit for the detection of **Tir**-producing *E. coli* are provided. A method

Searcher : Shears 308-4994

of immunizing a host with **Tir** to induce a protective immune response to **Tir** or a second **polypeptide** of interest is also provided. A method for screening for compds. which interfere with the **binding** of bacterial pathogens to their receptors is further provided. Thus, **protein Hp90**, previously believed to be a host membrane **protein**, has been identified as an **EHEC-** or **EPEC-secreted protein** which acts as an **intimin** receptor. **Proteins** encoded by the **espA** and **espB** genes were necessary for delivery of **Tir** to the host membrane.

IT 200662-09-5P 224307-15-7P

RL: ANT (Analyte); BOC (Biological occurrence); BPN (Biosynthetic preparation); BPR (Biological process); PRP (Properties); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process)

(amino acid sequence; pathogenic *Escherichia coli* **intimin** receptor **Tir** and gene **tir** and methods for detecting gene **tir** or **Tir protein** and for drug screening)

REFERENCE COUNT:

6

REFERENCE(S):

- (1) Deibel, C; Molecular Microbiology 1998, V28(3), P463 CAPLUS
- (2) Kenny, B; Cell 1997, V91, P511 CAPLUS
- (3) Kenny, B; Infection and Immunity 1997, V65(7), P2528 CAPLUS
- (4) Paton, A; Database EMBL - EMPRO 1998
- (5) Paton, A; Infection and Immunity 1998, V66(11), P5580 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 26 OF 34 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:291203 CAPLUS

DOCUMENT NUMBER: 131:85390

TITLE: **Enterohemorrhagic** *Escherichia*

coli O157:H7 produces **Tir**, which is translocated to the host cell membrane but is not tyrosine phosphorylated

AUTHOR(S): DeVinney, Rebekah; Stein, Markus; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; Finlay, B. Brett

CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z4, Can.

SOURCE: Infect. Immun. (1999), 67(5), 2389-2398

CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic *E. coli* (EPEC), this activity is dependent upon translocation of the intimin receptor, Tir, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a Tir-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional Tir that is inserted into host cell membranes, where it serves as an intimin receptor. However, unlike in EPEC, in EHEC Tir is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize Tir in Luria-Bertani medium but was able to secrete Tir into M9 medium, suggesting that Tir synthesis and secretion may be regulated differently in these two pathogens. EHEC Tir and EPEC Tir both bind intimin and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC intimins are functionally interchangeable, but EHEC Tir shows a much greater affinity for EHEC intimin than for EPEC intimin. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.

IT 212262-89-0

RL: PRP (Properties)

(amino acid sequence; enterohemorrhagic *Escherichia coli* O157:H7 produces Tir, which is translocated to host cell membrane but is not tyrosine phosphorylated)

REFERENCE COUNT:

45

REFERENCE(S):

- (1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
- (4) Bieber, D; Science 1998, V280, P2114 CAPLUS
- (5) Brunder, W; V Mol:Microbiol 1997, V24, P767 CAPLUS
- (6) Dean-Nystrom, E; Infect Immun 1998, V66, P4560 CAPLUS
- (7) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE CARLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO,
'KIN, TOXLIT, TOXLINE' ENTERED AT 12:00:10 ON 28 SEP 2001)

L11 1156 S FINLAY B?/AU
L12 350 S KENNY B?/AU
L13 62 S (DE VINNEY R? OR DEVINNEY R?)/AU
L14 4379 S STEIN M?/AU
L15 8 S L11 AND L12 AND L13 AND L14
L16 108 S L11 AND (L12 OR L13 OR L14)
L17 29 S L12 AND (L13 OR L14)
L18 12 S L13 AND L14
L19 5798 S L11 OR L12 OR L13 OR L14
L20 110 S (L16 OR L19) AND L4
L21 7 S L20 AND (PURE OR PURIF?)
39 S L15 OR L17 OR L18 OR L21

- Author(s)

REMOVED)

Searcher : Shears 308-4994

L23 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 2000:603715 CAPLUS
DOCUMENT NUMBER: 133:280269
TITLE: Human response to Escherichia coli O157:H7
infection: antibodies to secreted virulence
factors
AUTHOR(S): Li, Yuling; Frey, Elizabeth; Mackenzie, Andrew
M. R.; Finlay, B. Brett
CORPORATE SOURCE: Biotechnology Laboratory, University of British
Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Infect. Immun. (2000), 68(9), 5090-5095
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vaccination has been proposed for the prevention of disease due to enterohemorrhagic Escherichia coli (EHEC), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H7 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different EHEC virulence factors: **Tir** (translocated intimin receptor, which is inserted into the host cell membrane), intimin (bacterial outer membrane protein which binds to **Tir**), EspA (secreted protein which forms filamentous structures on EHEC surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examd. Sera were assayed against purified recombinant proteins using immunoblot anal. and by ELISA to det. the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and intimin in the acute-phase sera, although there was some reactivity to **Tir**. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against **Tir** (up to a titer of 1:256,000), esp. in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for **Tir**. These results suggest that there is a strong immune response to **Tir**, and to a lesser extent to the other three virulence factors, following EHEC disease, indicating that these bacterial mols. are potential vaccine candidates for preventing EHEC disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (**Tir** or EspB) are still recognized by the host immune response.

REFERENCE COUNT: 2

09/189415

REFERENCE(S) : (1) Abe, A; J Exp Med 1998, V188, P1907 CAPLUS
(2) Bitzan, M; J Clin Microbiol 1992, V30, P1174
MEDLINE

L23 ANSWER 2 OF 16 MEDLINE
ACCESSION NUMBER: 2000316068 MEDLINE
DOCUMENT NUMBER: 20316068 PubMed ID: 10858257
TITLE: Mechanical fractionation reveals structural
requirements for enteropathogenic Escherichia coli
Tir insertion into host membranes.
AUTHOR: Gauthier A; de Grado M; Finlay B B
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and
Biotechnology Laboratory, University of British
Columbia, Vancouver, British Columbia, V6T 1Z3,
Canada.
SOURCE: INFECTION AND IMMUNITY, (2000 Jul) 68 (7) 4344-8.
Journal code: GO7; 0246127. ISSN: 0019-9567..
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE).
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000720

AB Enteropathogenic Escherichia coli (EPEC) inserts its receptor for
intimate adherence (Tir) into host cell membranes by using
a type III secretion system. Detergents are frequently used to
fractionate infected host cells to investigate bacterial protein
delivery into mammalian cells. In this study, we found that the
Triton X-100-soluble membrane fraction from EPEC-infected HeLa cells
was contaminated with bacterial proteins. We therefore applied a
mechanical method of cell lysis and ultracentrifugation to
fractionate infected HeLa cells to investigate the biology and
biochemistry of Tir delivery and translocation. This
method demonstrates that the translocation of Tir into the
host cell membrane requires its transmembrane domains, but not
tyrosine phosphorylation or binding to Tir's ligand,
intimin.

L23 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 1999:577042 CAPLUS
DOCUMENT NUMBER: 131:194270
TITLE: Methods for assaying type III secretion
inhibitors
INVENTOR(S) : Finlay, Brett B.; Kenny, Brendan;
Stein, Marcus
PATENT ASSIGNEE(S) : University of British Columbia, Can.

Searcher : Shears 308-4994

09/189415

SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9945136	A1	19990910	WO 1999-CA183	19990305
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9932431	A1	19990920	AU 1999-32431	19990305
PRIORITY APPLN. INFO.:			US 1998-76980	P 19980305
			WO 1999-CA183	W 19990305

AB A method is provided for identifying compds. that specifically inhibit type III secretion systems that are used by several Gram-neg. animal and plant pathogens to secrete virulence factors that are crit. in causing disease. The compds. identified by this method are used as new antibacterial therapeutics. Specific inhibitors of the enteropathogenic Escherichia coli (EPEC) type III secretion system, that block EPEC signaling in host cells are identified by the use of specific mol. tools that have been developed with EPEC, including specific antibodies to secreted proteins and genetic fusions of epitope tags to genes encoding these secreted products. Promising compds. identified with the EPEC system are tested for their ability to inhibit type III secretion systems in other medically important pathogens.

REFERENCE COUNT: 2

REFERENCE(S): (1) Brett, F; WO 9740063 A 1997 CAPLUS
(2) Holden, D; WO 9617951 A 1996 CAPLUS

L23 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3

ACCESSION NUMBER: 1999:326051 CAPLUS

DOCUMENT NUMBER: 130:333761

TITLE: Pathogenic Escherichia coli intimin receptor Tir and gene tir and methods for detecting gene tir or Tir protein and for drug screening

INVENTOR(S): Finlay, B. Brett; Kenny, Brendan; Devinney, Rebekah; Stein, Marcus

Searcher : Shears 308-4994

09/189415

PATENT ASSIGNEE(S): University of British Columbia, Can.
 SOURCE: PCT Int. Appl., 91 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9924576	A1	19990520	WO 1998-CA1042	19981110
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9911373	A1	19990531	AU 1999-11373	19981110
EP 1029054	A1	20000823	EP 1998-954076	19981110
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1997-65130 P 19971112
 WO 1998-CA1042 W 19981110

AB A polypeptide, called Tir (for translocated intimin receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to Tir to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding Tir polypeptide. Isolated nucleic acid sequences encoding Tir polypeptide, Tir peptides, a recombinant method for producing recombinant Tir, antibodies which bind to Tir, and a kit for the detection of Tir-producing E. coli are provided. A method of immunizing a host with Tir to induce a protective immune response to Tir or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin receptor. Proteins encoded by the espA and espB genes were necessary for delivery of Tir to the host membrane.

REFERENCE COUNT: 6
 REFERENCE(S): (1) Deibel, C; Molecular Microbiology 1998, V28(3), P463 CAPLUS
 (2) Kenny, B; Cell 1997, V91, P511 CAPLUS
 (3) Kenny, B; Infection and Immunity 1997, V65(7), P2528 CAPLUS
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 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 5 OF 16 TOXLIT

ACCESSION NUMBER: 1999:65666 TOXLIT
 DOCUMENT NUMBER: CA-131-194270E
 TITLE: Methods for assaying type III secretion inhibitors.
 AUTHOR: Finlay BB; Kenny B; Stein M
 SOURCE: (1999). PCT Int. Appl. PATENT NO. 9945136 09/10/1999
 (University of British Columbia).
 CODEN: PIXXD2.
 PUB. COUNTRY: CANADA
 DOCUMENT TYPE: Patent
 FILE SEGMENT: CA
 LANGUAGE: English
 OTHER SOURCE: CA 131:194270
 ENTRY MONTH: 199910

AB A method is provided for identifying compds. that specifically inhibit type III secretion systems that are used by several Gram-neg. animal and plant pathogens to secrete virulence factors that are crit. in causing disease. The compds. identified by this method are used as new antibacterial therapeutics. Specific inhibitors of the enteropathogenic Escherichia coli (EPEC) type III secretion system, that block EPEC signaling in host cells are identified by the use of specific mol. tools that have been developed with EPEC, including specific antibodies to secreted proteins and genetic fusions of epitope tags to genes encoding these secreted products. Promising compds. identified with the EPEC system are tested for their ability to inhibit type III secretion systems in other medically important pathogens.

L23 ANSWER 6 OF 16 TOXLIT

ACCESSION NUMBER: 1999:23044 TOXLIT
 DOCUMENT NUMBER: CA-130-333761K
 TITLE: Pathogenic Escherichia coli intimin receptor Tir and gene tir and methods for detecting gene tir or Tir protein and for drug screening.
 AUTHOR: Finlay BB; Kenny B; Devinney R; Stein M
 SOURCE: (1999). PCT Int. Appl. PATENT NO. 9924576 05/20/1999

(University of British Columbia).

CODEN: PIXXD2.

PUB. COUNTRY: CANADA
 DOCUMENT TYPE: Patent
 FILE SEGMENT: CA
 LANGUAGE: English
 OTHER SOURCE: CA 130:333761
 ENTRY MONTH: 199906

AB A polypeptide, called Tir (for translocated intimin receptor), which is secreted by attaching and effacing pathogens, such as the enteropathogenic (EPEC) and enterohemorrhagic (EHEC) E. coli is disclosed. These bacterial pathogens insert their own receptors into mammalian cell surfaces, to which the bacterial pathogen then adheres to trigger addnl. host signaling events and actin nucleation. Diagnosis of disease caused by pathogenic E. coli can be performed by the use of antibodies which bind to Tir to detect the protein or the use of nucleic acid probes for detection of nucleic acids encoding Tir polypeptide. Isolated nucleic acid sequences encoding Tir polypeptide, Tir peptides, a recombinant method for producing recombinant Tir, antibodies which bind to Tir, and a kit for the detection of Tir-producing E. coli are provided. A method of immunizing a host with Tir to induce a protective immune response to Tir or a second polypeptide of interest is also provided. A method for screening for compds. which interfere with the binding of bacterial pathogens to their receptors is further provided. Thus, protein Hp90, previously believed to be a host membrane protein, has been identified as an EHEC- or EPEC-secreted protein which acts as an intimin receptor. Proteins encoded by the espA and espB genes were necessary for delivery of Tir to the host membrane.

L23 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
 ACCESSION NUMBER: 1999:291203 CAPLUS
 DOCUMENT NUMBER: 131:85390
 TITLE: Enterohemorrhagic Escherichia coli O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated
 AUTHOR(S): DeVinney, Rebekah; Stein, Markus; Reinscheid, Dieter; Abe, Akio; Ruschkowski, Sharon; Finlay, B. Brett
 CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z4, Can.
 SOURCE: Infect. Immun. (1999), 67(5), 2389-2398
 CODEN: INFIBR; ISSN: 0019-9567
 PUBLISHER: American Society for Microbiology
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Intimate attachment to the host cell leading to the formation of attaching and effacing (A/E) lesions is an essential feature of

enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 pathogenesis. In a related pathogen, enteropathogenic *E. coli* (EPEC), this activity is dependent upon translocation of the intimin receptor, Tir, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated proteins beneath adherent EHEC bacteria does not occur, leading to questions about whether EHEC uses a Tir-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that EHEC produces a functional Tir that is inserted into host cell membranes, where it serves as an intimin receptor. However, unlike in EPEC, in EHEC Tir is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. EHEC, but not EPEC, was unable to synthesize Tir in Luria-Bertani medium but was able to secrete Tir into M9 medium, suggesting that Tir synthesis and secretion may be regulated differently in these two pathogens. EHEC Tir and EPEC Tir both bind intimin and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. EHEC and EPEC intimins are functionally interchangeable, but EHEC Tir shows a much greater affinity for EHEC intimin than for EPEC intimin. These findings highlight some of the differences and similarities between EHEC and EPEC virulence mechanisms, which can be exploited to further define the mol. basis of pedestal formation.

REFERENCE COUNT:

45

REFERENCE(S):

- (1) Abe, A; Infect Immun 1997, V65, P3547 CAPLUS
- (4) Bieber, D; Science 1998, V280, P2114 CAPLUS
- (5) Brunder, W; V Mol:Microbiol 1997, V24, P767 CAPLUS
- (6) Dean-Nystrom, E; Infect Immun 1998, V66, P4560 CAPLUS
- (7) Deibel, C; Mol Microbiol 1998, V28, P463 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 8 OF 16 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998187833 EMBASE

TITLE: EPEC delivers the goods.

AUTHOR: Kaper J.B.; Finlay B.B.; DeVinney R.; Kenny B.; Stein M.

CORPORATE SOURCE: J.B. Kaper, Center for Vaccine Development, University of Maryland, School of Medicine, 685 West Baltimore St, Baltimore, MD 21201, United States. jkaper@umaryland.edu

SOURCE: Trends in Microbiology, (1998) 6/5 (169-172). Refs: 20

ISSN: 0966-842X CODEN: TRMIEA

PUBLISHER IDENT.: S 0966-842X(98)01266-9

COUNTRY: United Kingdom

09/189415

DOCUMENT TYPE: Journal; Note
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
048 Gastroenterology
LANGUAGE: English

L23 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5
ACCESSION NUMBER: 1997:717933 CAPLUS
DOCUMENT NUMBER: 128:33769
TITLE: Pathogenic Escherichia coli associated protein
EspA and espA gene encoding EspA
INVENTOR(S): Finlay, B. Brett; Stein, Markus;
Kenny, Brendan
PATENT ASSIGNEE(S): University of British Columbia, Can.; Finlay, B.
Brett; Stein, Markus; Kenny, Brendan
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9740063	A2	19971030	WO 1997-CA265	19970423
WO 9740063	A3	19980326		
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
CA 2252372	AA	19971030	CA 1997-2252372	19970423
AU 9725628	A1	19971112	AU 1997-25628	19970423
EP 904288	A2	19990331	EP 1997-917185	19970423
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
CN 1222937	A	19990714	CN 1997-195690	19970423
BR 9710433	A	19991005	BR 1997-10433	19970423
JP 2000511771	T2	20000912	JP 1997-537532	19970423
PRIORITY APPLN. INFO.:			US 1996-15999 P	19960423
			WO 1997-CA265 W	19970423

AB The present invention provides the EspA polypeptide, which is secreted by pathogenic E. coli, such as the enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC). Diagnosis of disease caused by such pathogenic E. coli can be performed by std.

Searcher : Shears 308-4994

techniques, such as those based upon the use of antibodies which bind to EspA to detect the protein, as well as those based on the use of nucleic acid probes for detection of nucleic acids encoding EspA protein. The invention also provides isolated nucleic acid sequences encoding EspA, EspA polypeptide, EspA peptides, a method for producing recombinant EspA, antibodies which bind to EspA, and a kit for the detection of EspA-producing *E. coli*. The invention also provides a method of immunizing a host with EspA to induce a protective immune response to EspA. DNA sequence of *espA* gene was analyzed, plasmid encoding a mutant *espA* gene was constructed, abolished EspA secretion and virulence of pathogenic *E. coli* by disrupting *espA* gene were obsd., and assay of screening inhibitors of bacterial type III secretion was developed.

L23 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 6
 ACCESSION NUMBER: 1997:596403 CAPLUS
 DOCUMENT NUMBER: 127:290298
 TITLE: Characterization of two virulence proteins
 secreted by rabbit enteropathogenic *Escherichia coli*, EspA and EspB, whose maximal expression is sensitive to host body temperature
 AUTHOR(S): Abe, Akio; Kenny, Brendan; Stein, Markus; Finlay, B. Brett
 CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
 SOURCE: Infect. Immun. (1997), 65(9), 3547-3555
 CODEN: INFIBR; ISSN: 0019-9567
 PUBLISHER: American Society for Microbiology
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Enteropathogenic *Escherichia coli* (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathol. features on intestinal mucosa; including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-1 has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified EPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the *espA* and *espB* genes were cloned and their sequences were compared to that of EPEC O127. The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-1 EspB was identical to that of enterohemorrhagic *E. coli* serotype O26. Mutations in RDEC-1 *espA* and *espB* revealed that the corresponding RDEC-1 gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids contg. EPEC

espA or/and espB genes into RDEC-1 mutant strains demonstrated that they were functionally interchangeable, although the EPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their resp. host body temps., which may contribute to the lack of EPEC infectivity in rabbits.

L23 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
 ACCESSION NUMBER: 1997:408582 CAPLUS
 DOCUMENT NUMBER: 127:119431
 TITLE: Enteropathogenic Escherichia coli protein secretion is induced in response to conditions similar to those in the gastrointestinal tract
 AUTHOR(S): Kenny, Brendan; Abe, Akio; Stein, Markus; Finlay, B. Brett
 CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T-1Z3, Can.
 SOURCE: Infect. Immun. (1997), 65(7), 2606-2612
 CODEN: INFIBR; ISSN: 0019-9567
 PUBLISHER: American Society for Microbiology
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The pathogenicity of enteropathogenic Escherichia coli (EPEC) is assocd. with the expression and secretion of specific bacterial factors. EspB is one such secreted protein which is required to trigger host signaling pathways resulting in effacement of microvilli and cytoskeletal rearrangements. These events presumably contribute to the ensuing diarrhea assocd. with EPEC infections. EPEC encounters several environmental changes and stimuli during its passage from the external environment into the host gastrointestinal tract. In this paper we show that the secretion of EspB is subject to environmental regulation, and maximal secretion occurs under conditions reminiscent of those in the gastrointestinal tract. Thus, secretion is maximal at 37.degree.C, pH 7, and physiol. osmolarity. In addn., maximal secretion requires the presence of sodium bicarbonate and calcium and is stimulated by millimolar concns. of Fe(NO3)3. The secretion of the four other EPEC-secreted proteins appears to be modulated in a manner similar to that of EspB. Our results also show that secretion is not dependent on CO2, as originally reported by Haigh et al., but that CO2 more likely acts as a component of the medium buffering system, since CO2 dependence was abolished by the use of alternative buffers.

L23 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8
 ACCESSION NUMBER: 1997:755652 CAPLUS
 DOCUMENT NUMBER: 128:72707
 TITLE: Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian

cells

AUTHOR(S) : Kenny, Brendan; DeVinney, Rebekah; Stein, Markus; Reinscheid, Dieter J.; Frey, Elizabeth A.; Finlay, B. Brett

CORPORATE SOURCE: Biotechnol. Lab., Dep. Biochem. Mol. Biol., Dep. Microbiol. Immunology, Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.

SOURCE: Cell (Cambridge, Mass.) (1997), 91(4), 511-520
CODEN: CELLB5; ISSN: 0092-8674

PUBLISHER: Cell Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enteropathogenic Escherichia coli (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence proteins needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a protein in the host membrane, Hp90, which is the receptor for the EPEC outer membrane protein, intimin. Hp90-intimin interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that Hp90 is actually a bacterial protein (Tir). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger addnl. host signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.

L23 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:187776 BIOSIS

DOCUMENT NUMBER: PREV199799486979

TITLE: Enteropathogenic E. coli exploitation of host epithelial cells.

AUTHOR(S) : Finlay, B. Brett (1); Ruschkowski, Sharon; Kenny, Brendan; Stein, Markus; Reinscheid, Dieter J.; Stein, Murry A.; Rosenshine, Ilan

CORPORATE SOURCE: (1) Biotechnol. Lab., Univ. B.C., Vancouver, BC V6T 1Z3 Canada

SOURCE: Ades, E. W. [Editor]; Morse, S. A. [Editor]; Rest, R. F. [Editor]. Annals of the New York Academy of Sciences, (1996) Vol. 797, pp. 26-31. Annals of the New York Academy of Sciences; Microbial pathogenesis and immune response, II.
Publisher: New York Academy of Sciences 2 East 63rd Street, New York, New York 10021, USA.
Meeting Info.: Conference New York, New York, USA
October 25-28, 1995

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ISSN: 0077-8923. ISBN: 1-57331-017-4 (paper),
1-57331-016-6 (cloth).
DOCUMENT TYPE: Book; Conference
LANGUAGE: English

L23 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 9
ACCESSION NUMBER: 1996:679649 CAPLUS
DOCUMENT NUMBER: 126:3760
TITLE: Characterization of EspC, a 110-kilodalton
protein secreted by enteropathogenic *Escherichia coli* which is homologous to members of the
immunoglobulin A protease-like family of
secreted proteins
AUTHOR(S): Stein, Markus; Kenny, Brendan
; Stein, Murry A.; Finlay, B. Brett
CORPORATE SOURCE: Dep. Biochem. Mol. Biol., Univ. British
Columbia, Vancouver, BC, V6T-1Z3, Can.
SOURCE: J. Bacteriol. (1996), 178(22), 6546-6554
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enteropathogenic *Escherichia coli* (EPEC) secretes .gtoreq.5
proteins. Two of these proteins, EspA and EspB (previously called
EaeB), activate signal transduction pathways in host epithelial
cells. While the role of the other three proteins (39, 40, and 110
kDa) remains undetd., secretion of all 5 proteins is under the
control of perA, a known pos. regulator of several EPEC virulence
factors. On the basis of N-terminal protein sequence data, we
cloned and sequenced the gene which encodes the 110-kDa secreted
protein and examd. its possible role in EPEC signaling and
interaction with epithelial cells. In accordance with the terminol.
used for espA and espB, we called this gene espC, for EPEC-secreted
protein C. We found significant homol. between the predicted EspC
protein sequence and a family of IgA (IgA) protease-like proteins
which are widespread among pathogenic bacteria. Members of this
protein family are found in avian pathogenic *Escherichia coli* (Tsh),
Haemophilus influenzae (Hap), and *Shigella flexneri* (SepA).
Although these proteins and EspC do not encode IgA protease
activity, they have considerable homol. with IgA protease from
Neisseria gonorrhoeae and *H. influenzae* and appear to use a export
system for secretion. We found that genes homologous to espC also
exist in other pathogenic bacteria which cause attaching and
effacing lesions, including *Hafnia alvei* biotype 19982, *Citrobacter*
freundii biotype 4280, and rabbit diarrheagenic *E. coli* (RDEC-1).
Although these strains secrete various proteins similar in mol. size
to the proteins secreted by EPEC, we did not detect secretion of a
110-kDa protein by these strains. To examine the possible role of

EspC in EPEC interactions with epithelial cells, we constructed a deletion mutant in espC by allelic exchange and characterized the mutant by std. tissue culture assays. We found that EspC is not necessary for mediating EPEC-induced signal transduction in HeLa epithelial cells and does not play a role in adherence or invasion of tissue culture cells.

L23 ANSWER 15 OF 16 MEDLINE DUPLICATE 10
 ACCESSION NUMBER: 97146492 MEDLINE
 DOCUMENT NUMBER: 97146492 PubMed ID: 8993348
 TITLE: Enteropathogenic E. coli exploitation of host epithelial cells.
 AUTHOR: Finlay B B; Ruschkowski S; Kenny B; Stein M; Reinscheid D J; Stein M A; Rosenshine I
 CORPORATE SOURCE: Biotechnology Laboratory, University of British Columbia, Vancouver, Canada.. bfinlay@unixg.ubc.ca
 SOURCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1996 Oct 25) 797 26-31. Ref: 51
 Journal code: 5NM; 7506858. ISSN: 0077-8923.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199701
 ENTRY DATE: Entered STN: 19970219
 Last Updated on STN: 19970219
 Entered Medline: 19970131

AB Enteropathogenic E. coli (EPEC) is a leading cause of neonatal diarrhea worldwide. These organisms adhere to the intestinal cell surface, causing rearrangement in the epithelial cell surface and underlying cytoskeleton, resulting in a structure termed an attaching/effacing (A/E) lesion. A/E lesion formation is thought necessary for EPEC-mediated disease. EPEC secretes several proteins that trigger signal transduction, intimate adherence, and cytoskeletal rearrangements in epithelial cells. Additionally, it produces intimin, an outer membrane product that mediates intimate adherence. Together these various bacterial molecules contribute to the intimate relationship that is formed by EPEC with host epithelial cells which results in A/E lesion formation and diarrhea.

L23 ANSWER 16 OF 16 MEDLINE
 ACCESSION NUMBER: 93010945 MEDLINE
 DOCUMENT NUMBER: 93010945 PubMed ID: 1396556
 TITLE: Signal transduction between enteropathogenic Escherichia coli (EPEC) and epithelial cells: EPEC

09/189415

induces tyrosine phosphorylation of host cell proteins to initiate cytoskeletal rearrangement and bacterial uptake.

AUTHOR: Rosenshine I; Donnenberg M S; Kaper J B; Finlay B B
CORPORATE SOURCE: Department of Biochemistry, University of British Columbia, Vancouver, Canada.
SOURCE: EMBO JOURNAL, (1992 Oct) 11 (10) 3551-60.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19980206
Entered Medline: 19921102

AB Upon attachment to cultured HeLa cells, enteropathogenic Escherichia coli (EPEC) induces assembly of a complex cytoskeletal structure within the eucaryotic cell, localized beneath the adherent bacterium. In addition, EPEC induces its own internalization by non-phagocytic epithelial cells. We found that after binding to the epithelial cell surface, EPEC induces tyrosine phosphorylation of three eucaryotic proteins. The major phosphorylation substrate is a 90 kDa protein (Hp90). In correlation with Hp90 tyrosine phosphorylation, the EPEC-induced cytoskeletal structure also contained tyrosine phosphorylated proteins. Using tyrosine protein kinase inhibitors and EPEC mutants (cfm) that fail to induce Hp90 phosphorylation, we demonstrate that induction of Hp90 phosphorylation is involved in initiation of the cytoskeletal structure assembly and in bacterial uptake. Other non-invasive EPEC mutants (eae) are still able to induce Hp90 tyrosine phosphorylation and to initiate aggregation of the tyrosine phosphorylated proteins and some cytoskeleton components. However, eae mutants are deficient in nucleating the aggregates into an organized structure.

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transform. 2 enteropathogenic strains of Escherichia coli to test for inhibitors. (51pp)

22/3,AB/12 (Item 2 from file: 357)

DIALOG(R)File 357:Derwent Biotechnology Abs
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0219264 DBA Accession No.: 98-00861 PATENT

EspA from enteropathogenic or enterohemorrhagic Escherichia coli - vector expression in host cell for recombinant protein production for use as a recombinant vaccine

AUTHOR: Finlay B B; *Stein M***; *Kenny B***

CORPORATE SOURCE: Vancouver, British Columbia, Canada.

PATENT ASSIGNEE: Univ.British-Columbia 1997

PATENT NUMBER: WO 9740063 PATENT DATE: 971030 WPI ACCESSION NO.:
97-535772 (9749)

PRIORITY APPLIC. NO.: US 15999 APPLIC. DATE: 960423

NATIONAL APPLIC. NO.: WO 97CA265 APPLIC. DATE: 970423

LANGUAGE: English

ABSTRACT: A new secreted EspA protein from Escherichia coli with a mol.wt. of 25,000 by SDS-PAGE is encoded by DNA (protein and DNA sequence specified) which can be contained on a vector and used to transform a host cell for production of the recombinant protein. Also claimed is an polyclonal or monoclonal antibody which binds to the EspA protein and which can be used to detect EspA in a tissue or biological fluid sample. The presence of EspA indicates infection by enteropathic E. coli. The protein may be used to immunize a host against disease caused by EspA-producing E. coli, or ameliorating such a disease. A DNA probe that hybridizes to the espA nucleic acid molecule can be used to detect espA in a sample. Also claimed is a method of identifying a compound which inhibits bacterial type-II secretion systems, a method for producing a nonpathogenic organism, preferably E. coli, and a method of producing a fusion protein containing EspA and a target protein. (62pp)
? ds; log y

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PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V32, N1 (APR), P151-158
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE,
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ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (EPEC) induce characteristic attaching and effacing (A/E) lesions on epithelial cells. This event is mediated, in part, by binding of the bacterial outer membrane protein, *intimin***, to a second EPEC protein, *Tir*** (*translocated*** *intimin*** receptor), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study, we have localized the *intimin***-binding domain of *Tir*** to a central 107-amino-acid region, designated *Tir***-M. We provide evidence that both the amino- and carboxy-termini of *Tir*** are located within the host cell. In addition, using immunogold labelling electron microscopy, we have confirmed that *intimin*** can bind independently to host cells even in the absence of *Tir***. This *Tir***-independent interaction and the ability of EPEC to induce A/E lesions requires an intact lectinlike module residing at the carboxy-terminus of the *intimin*** polypeptide. Using the yeast two-hybrid system and gel overlays, we show that *intimin*** can bind both *Tir*** and *Tir***-M even when the lectin-like domain is disrupted. These data provide strong evidence that *intimin*** interacts not only with *Tir*** but also in a lectinlike manner with a host cell *intimin*** receptor.

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10430360 GENUINE ARTICLE#: 182MT NUMBER OF REFERENCES: 46

TITLE: Structure of the cell-adhesion fragment of *intimin*** from
*enteropathogenic*** Escherichia *coli***

AUTHOR(S): Kelly G; Prasannan S; Daniell S; Fleming K; Frankel G; Dougan G;
Connerton I; Matthews S (REPRINT)

AUTHOR(S) E-MAIL: s.j.matthews@ic.ac.uk

CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept
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Wellcome Ctr Infect Dis, /London SW7 2AY//England/

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ISSN: 1072-8368

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia coli*** (EPEC) induce gross cytoskeletal rearrangement within epithelial cells, immediately beneath the attached bacterium. The C-terminal 280 amino acid residues of *intimin*** (Int280; 30.1 kDa), a bacterial cell-adhesion molecule, mediate the intimate bacterial host-cell interaction. Recently, interest in this process has been stimulated by the discovery that the bacterial *intimin*** receptor protein (*Tir***) is translocated into the host cell membrane, phosphorylated, and after binding *intimin*** triggers the intimate attachment. Using multidimensional nuclear magnetic resonance (NMR) and combining perdeuteration with site-specific protonation of methyl groups, we have determined the global fold of Int280. This represents one of the largest, non-oligomeric protein structures to be determined by NMR that has not been previously resolved by X-ray crystallography. Int280 comprises three domains; two immunoglobulin-like domains and a C-type lectinlike module, which define a new family of bacterial adhesion molecules. These findings also imply that carbohydrate recognition may be important in *intimin***-mediated cell adhesion.

ISSN: 1072-8368

6/3,AB/47 (Item 17 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10323436 GENUINE ARTICLE#: 170EB NUMBER OF REFERENCES: 33

TITLE: Phosphorylation of tyrosine 474 of the *enteropathogenic*** Escherichia coli*** (EPEC) *Tir*** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications

AUTHOR(S): Kenny B (REPRINT)

AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk

CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/

PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V31, N4 (FEB), P1229-1241

PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND

ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The *enteropathogenic*** Escherichia coli*** (EPEC) *Tir*** protein becomes tyrosine phosphorylated in host cells and displays an increase in apparent molecular mass. The interaction of *Tir*** with the EPEC outer membrane protein, *intimin***, triggers actin nucleation beneath the adherent bacteria. The *enterohaemorrhagic*** E. coli*** 0157:H7 (*EHEC***) *Tir*** molecule is not tyrosine phosphorylated. In

Searcher : Shears 308-4994

this paper, *Tir*** tyrosine phosphorylation is shown to be essential for actin nucleation activity, but not for the increase in apparent molecular mass observed in target cells. Tyrosine phosphorylation had no role in *Tir*** molecular mass shift, indicating additional host modifications. Analysis of *Tir*** intermediates indicates that tyrosine-independent modification functions to direct *Tir***'s correct insertion from the cytoplasm into the host membrane. Deletion analysis identified *Tir*** domains participating in translocation, association with the host membrane, modification and antibody recognition. *Intimin*** was found to bind a 55-amino-acid region (TIBA) within *Tir*** that topological and sequence analysis suggests is located in an extracellular loop. Homologous TIBA sequences exist in integrins, which also bind *intimin***. Collectively, this study provides definitive evidence for the importance of tyrosine phosphorylation for EPEC *Tir*** function and reveals differences in the pathogenicity of EPEC and *EHEC***. The data also suggest a mechanism for *Tir*** insertion into the host membrane, as well as providing clues to the mode of *intimin***-integrin interaction.

ISSN: 0950-382X

6/3,AB/48 (Item 18 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)
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10270099 GENUINE ARTICLE#: 165AC NUMBER OF REFERENCES: 32

TITLE: Detection of shiga-like toxin (stx(1) and stx(2)), *intimin*** (*eaeA***), and *enterohemorrhagic*** Escherichia *coli*** (*EHEC***) hemolysin (*EHEC*** hlyA) genes in animal feces by multiplex PCR

AUTHOR(S): Fagan PK; Hornitzky MA; Bettelheim KA; Djordjevic SP (REPRINT)

AUTHOR(S) E-MAIL: steve.djordjevic@agric.nsw.gov.au

CORPORATE SOURCE: New S Wales Agr, Elizabeth Macarthur Agr Inst, Private Mail Bag 8/Camden/NSW 2570/Australia/ (REPRINT); New S Wales Agr, Elizabeth Macarthur Agr Inst, /Camden/NSW 2570/Australia/; Fairfield Hosp, Victorian Infect Dis Reference Lab, /Fairfield/Vic 3078/Australia/

PUBLICATION TYPE: JOURNAL

PUBLICATION: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 1999, V65, N2 (FEB), P 868-872

PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171 USA

ISSN: 0099-2240

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: A multiplex PCR was developed for the rapid detection of genes encoding Shiga toxins 1 and 2 (stx(1) and stx(2)), *intimin*** (*eaeA***), and enterohemolysin A (hlyA) in 444 fecal samples derived from healthy and clinically affected cattle, sheep, pigs, and goats. The method involved non-solvent-based extraction of nucleic acid from an aliquot of an overnight culture of feces in EC (modified) broth. The

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detection limit of the assay for both fecal samples and pure cultures was between 18 and 37 genome equivalents. stx(1) and hlyA were the most commonly encountered virulence factors.

ISSN: 0099-2240

6/3,AB/49 (Item 19 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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10198343 GENUINE ARTICLE#: 159TW NUMBER OF REFERENCES: 28

TITLE: Virulence genes of Shiga toxin-producing Escherichia coli isolated from food, animals and humans

AUTHOR(S): Meng JH (REPRINT); Zhao SH; Doyle MP

AUTHOR(S) E-MAIL: jm332@umail.umd.edu

CORPORATE SOURCE: Univ Maryland, Dept Nutr & Food Sci, /College
Pk//MD/20742 (REPRINT); Univ Maryland, Dept Nutr & Food Sci, /College
Pk//MD/20742; Univ Georgia, Ctr Food Safety & Qual Enhancement,
/Griffin//GA/30223; Univ Georgia, Dept Food Sci & Technol,
/Griffin//GA/30223

PUBLICATION TYPE: JOURNAL

PUBLICATION: INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 1998, V45, N3 (DEC 22), P229-235

PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS

ISSN: 0168-1605

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The presence of virulence genes, encoding *enterohemorrhagic*** Escherichia *coli*** (*EHEC***)-hemolysin (*EHEC***-hlyA), *intimin*** (eae), and Shiga toxins 1 (stx1) and 2 (stx2), in 178 isolates of pathogenic E. *coli***, was determined using the polymerase chain reaction with primers specific for each virulence gene. The tested organisms were 120 isolates of E. coli O157:H7 from human patients, cattle, sheep and foods, 16 non-O157:H7 *EHEC*** isolates from patients suffering from hemorrhagic colitis or hemolytic uremic syndrome, 15 non-O157:H7 Shiga toxin-producing E. *coli*** (STEC) isolates from cattle and foods, 26 isolates of *enteropathogenic*** E. *coli*** (EPEC), enteroinvasive E. *coli*** (EIEC) and enterotoxigenic E. *coli*** (ETEC), and an E. *coli*** K12 strain. Results revealed that all isolates of O157:H7 carried *EHEC***-hlyA, eae, and one or both stx genes; 15 of the 16 non-O157:H7 *EHEC*** isolates had *EHEC***-hlyA, but all possessed eae and one or both stx genes; only seven of the 15 non-O157 STEC isolated from cattle and foods contained both *EHEC***-hlyA and eae genes. The EPEC, EIEC, ETEC, and the E. *coli*** K12 strain did not carry these virulence genes, except eight EPEC isolates were positive for eae. Results suggest that a combination of *EHEC***-hlyA and eae genes could serve as markers to differentiate *EHEC*** from less pathogenic STEC, and other pathogenic or non-pathogenic E. *coli***. (C) 1998 Elsevier Science B.V. All rights reserved.

ISSN: 0168-1605

Searcher : Shears 308-4994

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6/3,AB/50 (Item 20 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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09901899 GENUINE ARTICLE#: 125WL NUMBER OF REFERENCES: 21
TITLE: The medium is the messenger
AUTHOR(S): Phillips AD (REPRINT)
CORPORATE SOURCE: UNIV LONDON ROYAL FREE HOSP,DEPT PAEDIAT GASTROENTEROL,
POND ST/LONDON NW3 2QG//ENGLAND/ (REPRINT)
PUBLICATION TYPE: JOURNAL
PUBLICATION: GUT, 1998, V43, N4 (OCT), P456-457
PUBLISHER: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE,
TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND
ISSN: 0017-5749
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (EPEC), like many bacterial pathogens, employ a type III secretion system to deliver effector proteins across the bacterial cell, in EPEC, four proteins are known to be exported by a type III secretion system-EspA, EspB and EspD required for subversion of host cell signal transduction pathways and a *translocated*** *intimin*** receptor (*Tir***) protein (formerly Hp90) which is tyrosine-phosphorylated following transfer to the host cell to become a receptor for *intimin***-mediated intimate attachment and 'attaching and effacing' (A/E) lesion formation. The structural basis Mr protein translocation has yet to be fully elucidated for ally type ill secretion system. Here, we describe a novel EspA-containing filamentous organelle that is present on the bacterial surface during the early stage of A/E lesion formation, forms a physical bridge between the bacterium and the infected eukaryotic cell surface and is required for the translocation Of EspB into infected epithelial cells.
ISSN: 0017-5749

6/3,AB/51 (Item 21 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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09806492 GENUINE ARTICLE#: 115XX NUMBER OF REFERENCES: 7
TITLE: BipA affects Ca++ fluxes and phosphorylation of the *translocated*** *intimin*** receptor (*Tir***/Hp90) in host epithelial cells infected with *enteropathogenic*** E-*coli***
AUTHOR(S): Farris M (REPRINT); Grant A; Jane S; Chad J; OConnor CD
CORPORATE SOURCE: UNIV SOUTHAMPTON,SCH BIOL SCI, DIV BIOCHEM & MOL
BIOL/SOUTHAMPTON SO16 7PX/HANTS/ENGLAND/ (REPRINT); UNIV
SOUTHAMPTON,SCH BIOL SCI, DIV CELL SCI/SOUTHAMPTON SO16
7PX/HANTS/ENGLAND/
PUBLICATION TYPE: JOURNAL

Searcher : Shears 308-4994

09/189415

PUBLICATION: BIOCHEMICAL SOCIETY TRANSACTIONS, 1998, V26, N3 (AUG), P
S225-S225

PUBLISHER: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND

ISSN: 0300-5127

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ISSN: 0300-5127

6/3,AB/52 (Item 22 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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09417845 GENUINE ARTICLE#: ZJ871 NUMBER OF REFERENCES: 44

TITLE: A novel EspA-associated surface organelle of *enteropathogenic**
Escherichia *coli*** involved in protein translocation into epithelial
cells

AUTHOR(S): Knutton S (REPRINT); Rosenshine I; Pallen MJ; Nisan I; Neves BC;
Bain C; Wolff C; Dougan G; Frankel G

CORPORATE SOURCE: UNIV BIRMINGHAM, INST CHILD HLTH/BIRMINGHAM B16 8ET/W
MIDLANDS/ENGLAND/ (REPRINT); UNIV LONDON IMPERIAL COLL SCI TECHNOL &
MED, DEPT BIOCHEM/LONDON SW7 2AZ//ENGLAND/; HEBREW UNIV JERUSALEM, FAC
MED, DEPT MOL GENET & BIOTECHNOL/IL-91120 JERUSALEM//ISRAEL/; HEBREW
UNIV JERUSALEM, FAC MED, DEPT CLIN MICROBIOL/IL-91120 JERUSALEM//ISRAEL/

PUBLICATION TYPE: JOURNAL

PUBLICATION: EMBO JOURNAL, 1998, V17, N8 (APR 15), P2166-2176

PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP

ISSN: 0261-4189

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic** Escherichia *coli*** (EPEC), like among
bacterial pathogens, employ a type WI secretion system to deliver
effector proteins across the bacterial cell, In EPEC, four proteins are
known to be exported by a type III secretion system-EspA, EspB and EspD
required for subversion of host cell signal transduction pathways and a
*translocated*** *intimin*** receptor (*Tir***) protein (formerly Hp90)
which is tyrosine-phosphorylated following transfer to the host cell to
become a receptor for *intimin***-mediated intimate attachment and
'attaching and effacing' (A/E) lesion formation, The structural basis
for protein translocation has yet to be fully elucidated for any type
III secretion system. Here, we describe a novel EspA-confirming
filamentous organelle that is present on the bacterial surface during
the early stage of A/E lesion formation, forms a physical bridge
between the bacterium and the infected eukaryotic cell surface and is
required for the translocation of EspB into infected epithelial cells.

ISSN: 0261-4189

6/3,AB/53 (Item 23 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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Searcher : Shears 308-4994

09/189415

08996050 GENUINE ARTICLE#: YG492 NUMBER OF REFERENCES: 31

TITLE: *Enteropathogenic*** E-*coli*** (EPEC) transfers its receptor for intimate adherence into mammalian cells

AUTHOR(S): Kenny B; DeVinney R; Stein M; Reinscheid DJ; Frey EA; Finlay BB (REPRINT)

CORPORATE SOURCE: UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/

PUBLICATION TYPE: JOURNAL

PUBLICATION: CELL, 1997, V91, N4 (NOV 14), P511-520

PUBLISHER: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138

ISSN: 0092-8674

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** E. *coli*** (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence proteins needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a protein in the host membrane, Hp90, which is the receptor for the EPEC outer membrane protein, *intimin***. Hp90-*intimin** interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that Hp90 is actually a bacterial protein (*Tir***). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger additional host signaling events and actin nucleation. It is also tyrosine-phosphorylated upon transfer into the host cell.

ISSN: 0092-8674

6/3, AB/54 (Item 24 from file: 440)

DIALOG(R) File 440: Current Contents Search(R)

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08878118 GENUINE ARTICLE#: BJ63E NUMBER OF REFERENCES: 0

TITLE: Host exposure to *intimin*** (*EaeA***) prior to infection with an attaching/effacing rabbit enteropathogen - Is *intimin*** a protective antigen?

AUTHOR(S): Agin TS (REPRINT); Noel JM; McQueen CE; Boedeker EC; Wolf MK; Keusch GT; Kawakami M

CORPORATE SOURCE: WALTER REED ARMY MED CTR, WALTER REED ARMY INST RES/WASHINGTON//DC/20307 (REPRINT)

PUBLICATION TYPE: BOOK

PUBLICATION: CYTOKINES, CHOLERA, AND THE GUT, 1995, P315-320

PUBLISHER: I O S PRESS, VAN DIEMENSTRAAT 94, 1013 CN AMSTERDAM, NETHERLANDS

ISBN: 90-5199-298-X LIBRARY OF CONGRESS ID: 96-78112

LANGUAGE: English DOCUMENT TYPE: ARTICLE

Searcher : Shears 308-4994

09/189415

ABSTRACT: Bacteria require the *intimin*** (*EaeA***) protein to form attaching and effacing (A/E) lesions, characteristic of EPEC and *EHEC*** infections in humans and RDEC-1 infections in rabbits, on intestinal epithelia. We retrospectively analyzed rabbit sera for the presence of anti-*intimin*** IgG prior to infection with RDEC-1 or a derivative of RDEC-1 that expresses SLT-1, to determine if there was a correlation between prior exposure to *intimin*** and protection from disease. Five rabbits, three challenged with RDEC-1 and two challenged with RDEC-H19A, had pre-existing anti-*intimin*** IgG prior to challenge and all five animals showed good weight gain post challenge. Thirty-two rabbits lacked pre-existing anti-*intimin*** Ige and showed varying weight gain post challenge. These results suggest that the rabbits with prior exposure to *intimin*** were unlikely to be in the group exhibiting symptoms of RDEC-1 infection.

6/3,AB/55 (Item 25 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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07356412 GENUINE ARTICLE#: UJ557 NUMBER OF REFERENCES: 51
TITLE: ESPA, A PROTEIN SECRETED BY *ENTEROPATHOGENIC*** ESCHERICHIA *COLI***, IS REQUIRED TO INDUCE SIGNALS IN EPITHELIAL CELLS
AUTHOR(S): KENNY B; LAI LC; FINLAY BB; DONNENBERG MS (Reprint)
CORPORATE SOURCE: UNIV MARYLAND, SCH MED, DIV INFECT DIS, 10 S PINE ST, MSTF-900/BALTIMORE//MD/21201 (Reprint); UNIV MARYLAND, SCH MED, DIV INFECT DIS/BALTIMORE//MD/21201; UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOLEC BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA, DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/
PUBLICATION: MOLECULAR MICROBIOLOGY, 1996, V20, N2 (APR), P313-323
ISSN: 0950-382X
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (EPEC) is a leading cause of infant diarrhoea. EPEC mediates several effects on host epithelial cells, including activation of signal-transduction pathways, cytoskeletal rearrangement along with pedestal and attaching/effacing lesion formation. It has been previously shown that the EPEC eaeB (espB) gene encodes a secreted protein required for signal transduction and adherence, while *eaeA*** encodes *intimin***, an EPEC membrane protein that mediates intimate adherence and contributes to focusing of cytoskeletal proteins beneath bacteria. DNA-sequence analysis of a region between *eaeA*** and eaeB identified a predicted open reading frame (espA) that matched the amino-terminal sequence of a 25 kDa EPEC secreted protein. A mutant with a non-polar insertion in espA does not secrete this protein, activate epithelial cell signal transduction or cause cytoskeletal rearrangement. These phenotypes were complemented by a cloned espA gene. The espA mutant is also defective for invasion, It is concluded that espA encodes an EPEC secreted protein that is necessary for activating epithelial signal transduction, intimate

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contact, and formation of attaching and effacing lesions, processes which are central to pathogenesis.

ISSN: 0950-382X

6/3,AB/56 (Item 26 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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06508323 GENUINE ARTICLE#: RE529 NUMBER OF REFERENCES: 27
TITLE: IDENTIFICATION OF *EAEA*** PROTEIN IN THE OUTER MEMBRANE OF
ATTACHING AND EFFACING ESCHERICHIA COLI 045 FROM PIGS
AUTHOR(S): ZHU CR; HAREL J; DUMAS F; FAIRBROTHER JM (Reprint)
CORPORATE SOURCE: UNIV MONTREAL,FAC VET MED,RECH MALAD INFECT PORC GRP,CP
5000/ST HYACINTHE/PQ J2S 7C6/CANADA/ (Reprint); UNIV MONTREAL,FAC VET
MED,RECH MALAD INFECT PORC GRP/ST HYACINTHE/PQ J2S 7C6/CANADA/; NATL
RES COUNCIL CANADA,BIOTECHNOL RES INST/MONTREAL/PQ H4P 2R2/CANADA/
PUBLICATION: FEMS MICROBIOLOGY LETTERS, 1995, V129, N2-3 (JUN 15), P237-242
ISSN: 0378-1097
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: We have previously reported that the production of attaching and effacing lesions by Escherichia *coli*** 045 isolates from pigs is associated with the *eaeA*** (E. *coli*** attaching and effacing) gene. In the present study, expression of the *EaeA*** protein, the *eaeA*** gene product, among swine 045 E. *coli*** isolates was examined. The majority (20/22) of attaching and effacing positive, *eaeA***(+) E. *coli*** 045 isolates, but none of ten attaching and effacing negative, *eaeA***(-) or *eaeA***(+) isolates, expressed a 97-kDa outer membrane protein as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (*SDS***-PAGE***) and Western blot analysis. Amino-terminal amino acid sequencing demonstrated a high homology between this 97-kDa protein of swine E. *coli*** 045 and the *EaeA*** protein (*intimin***) of human *enteropathogenic*** E. *coli*** and *enterohemorrhagic*** E. *coli***. In addition, a serological relationship between the *EaeA*** proteins of swine 045, rabbit (RDEC-1) and human (E2348/69) attaching and effacing E. *coli*** strains was observed. Our results indicate an association between expression of the *EaeA*** protein and attaching and effacing activity among 045 E. *coli*** isolates. The data also suggest an antigenic relatedness of the *EaeA*** proteins of swine, rabbit, and human attaching and effacing E. *coli***.

ISSN: 0378-1097

6/3,AB/57 (Item 27 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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04739646 GENUINE ARTICLE#: LP331 NUMBER OF REFERENCES: 45
Searcher : Shears 308-4994

09/189415

TITLE: A 2ND CHROMOSOMAL GENE NECESSARY FOR INTIMATE ATTACHMENT OF
*ENTEROPATHOGENIC*** ESCHERICHIA-*COLI*** TO EPITHELIAL CELLS

AUTHOR(S): DONNENBERG MS; YU J; KAPER JB

CORPORATE SOURCE: DEPT VET AFFAIRS MED CTR, MED SERV/BALTIMORE//MD/21201
(Reprint); UNIV MARYLAND, SCH MED, DEPT MED, CTR VACCINE
DEV/BALTIMORE//MD/21201; UNIV MARYLAND, SCH MED, DIV INFECT
DIS/BALTIMORE//MD/21201

PUBLICATION: JOURNAL OF BACTERIOLOGY, 1993, V175, N15 (AUG), P4670-4680
ISSN: 0021-9193

LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (EPEC) is capable of
attaching intimately to epithelial cells and effacing their microvilli.
A chromosomal locus, *eaeA*** (originally eae), is required for the
intimate attachment aspect of this effect. We report the mapping of a
region of the EPEC chromosome that is located immediately downstream of
the *eaeA*** gene and that is also necessary for intimate attachment.
An isogenic in-frame deletion mutation in one of the open reading
frames identified in this region was engineered. Because the resulting
mutant, like an *eaeA*** deletion mutant, is deficient in the ability
to attach intimately to epithelial cells, the mutated gene is
designated eaeB. Full activity is restored to the eaeB mutant when the
cloned gene is reintroduced on a plasmid. The eaeB mutant remains
capable of producing *intimin***, the product of the *eaeA*** gene. No
differences in the fractionation properties or electrophoretic mobility
of *intimin*** are apparent in the eaeB mutant. The product of the eaeB
locus was identified by in vitro transcription-translation. The
nucleotide sequence of the eaeB gene predicts a protein that contains a
sequence motif common to several aminotransferase enzymes. These
results indicate that the attaching and effacing effect is a complex
phenotype dependent on a gene cluster present on the EPEC chromosome.

ISSN: 0021-9193

6/3, AB/58 (Item 1 from file: 348)

DIALOG(R) File 348: European Patents

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00954338

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Non-pathogenic E.Coli mutant strain, process or their preparation and uses
Nicht pathogener mutanter E. coli Stamm, Verfahren zur dessen Herstellung
und Verwendung

Souches mutantes non pathogenes d'E.coli, leur procede d'obtention et leurs
utilisations

PATENT ASSIGNEE:

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states: BE; DE; ES; FR; IT)

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Searcher : Shears 308-4994

09/189415

Capelles, 31076 Toulouse Cedex, (FR), (applicant designated states:
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INVENTOR:

Milon, Alain, 13 avenue du 11 Novembre 1918, 31700 Blagnac, (FR)

De Rycke, Jean, 12 chemin de Larriou, 31820 Pibrac, (FR)

LEGAL REPRESENTATIVE:

Vialle-Presles, Marie Jose et al (75731), Cabinet Ores, 6, Avenue de
Messine, 75008 Paris, (FR)

PATENT (CC, No, Kind, Date): EP 864644 A1 980916 (Basic)

APPLICATION (CC, No, Date): EP 98400093 980120;

PRIORITY (CC, No, Date): FR 97532 970120

DESIGNATED STATES: BE; DE; ES; FR; IT

INTERNATIONAL PATENT CLASS: C12N-001/20

ABSTRACT EP 864644 A1 (Translated)

Non-pathogenic Escherichia coli mutants lacking cytopathic phenotype

Non-pathogenic E. coli mutants are produced from a pathogenic strain
(cytopathic for HeLa cells and able: (a) to induce formation of actin
filaments right through the cells, and (b) to increase the level of
vinculin) by mutagenesis and selection for loss of cytopathic capacity.

TRANSLATED ABSTRACT WORD COUNT: 51

ABSTRACT EP 864644 A1

L'invention concerne un procede d'obtention de souches mutantes
non-pathogenes d'E. coli, a partir de souches pathogenes capables
d'induire sur des cellules epitheliales HeLa un effet cytopathique se
manifestant par la formation de cables d'actine polymerisee traversant de
part en part lesdites cellules et par une augmentation de la quantite de
vinculine.

L'invention concerne egalement les souches mutantes non-pathogenes
susceptibles d'etre obtenues par ce procede, et leur utilisation
vaccinale.

ABSTRACT WORD COUNT: 69

LANGUAGE (Publication,Procedural,Application): French; French; French

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(French)	9838	315
SPEC A	(French)	9838	6654
Total word count - document A			6969
Total word count - document B			0
Total word count - documents A + B			6969

6/3,AB/59 (Item 1 from file: 357)

DIALOG(R) File 357:Derwent Biotechnology Abs

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0239735 DBA Accession No.: 1999-09836 PATENT

Searcher : Shears 308-4994

09/189415

Escherichia coli recombinant *intimin*** receptor protein - useful for distinguishing between enteropathogenic and enterohemorrhagic infection and for therapy and diagnosis

AUTHOR: Finlay B B; Kenny B; Devinney R; Stein M

CORPORATE SOURCE: Vancouver, British Columbia, Canada.

PATENT ASSIGNEE: Univ.British-Columbia 1999

PATENT NUMBER: WO 9924576 PATENT DATE: 19990520 WPI ACCESSION NO.: 1999-337712 (1928)

PRIORITY APPLIC. NO.: US 65130 APPLIC. DATE: 19971112

NATIONAL APPLIC. NO.: WO 98CA1042 APPLIC. DATE: 19981110

LANGUAGE: English

ABSTRACT: A translocated Escherichia *coli*** *intimin*** receptor protein

(I) that binds *intimin*** is new. Also claimed are: a DNA sequence

(II) encoding (I) and its complements, fragments and variants; DNA

probes specific for (II); vectors encoding (II) and host cells

containing them; (I)-specific polyclonal or monoclonal antibody;

recombinant production of (I); a fusion protein containing (I); a

method for identifying modulators of (I); a method for differentiating

between attaching and effacing pathogens by contacting them with an

anti-(I) antibody and an anti-phosphotyrosine antibody; drug delivery

to (I)-containing cells using a cell delivery vehicle; kits for the

detection of (I) and (II); and a method for inducing a cell-mediated

immune response in cattle or humans to a protein of interest by

contacting a subject with an attenuated bacteria, where the bacterium

lacks an EspA or EspB protein, and contains (II) in a fusion construct.

The presence of (I) in a sample is indicative of *enteropathogenic***

or *enterohemorrhagic*** infection. (91pp)

Set	Items	Description
S7	110	S1 AND PATHOGEN? ?
S8	39	S7 AND (INTIMIN OR SDSPAGE OR SDS(W) PAGE)
S9	6	S8 NOT S5
S10	5	RD (unique items)

? t 10/3,ab/1-5

>>>No matching display code(s) found in file(s): 65, 113

10/3,AB/1 (Item 1 from file: 144)

DIALOG(R)File 144:Pascal

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14451724 PASCAL No.: 00-0110706

Rapid and sensitive detection of Escherichia coli O157:H7 in bovine faeces by a multiplex PCR

HU Y; ZHANG Q; MEITZLER J C

Food Animal Health Research Program, Department of Veterinary Preventive Medicine, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, United States

Journal: Journal of applied microbiology, 1999, 87 (6) 867-876

Searcher : Shears 308-4994

09/189415

Language: English

Cattle are considered the major reservoir for *Escherichia coli* O157:H7, one of the newly emerged foodborne human *pathogens*** of animal origin and a leading cause of haemorrhagic colitis in humans. A sensitive test that can accurately and rapidly detect the organism in the food animal production environment is critically needed to monitor the emergence, transmission, and colonization of this *pathogen*** in the animal reservoir. In this study, a novel multiplex polymerase chain reaction (PCR) assay was developed by using 5 sets of primers that specifically amplify segments of the *eaeA***, *slt-I*, *slt-II*, *fliC*, *rfbE* genes, which allowed simultaneous identification of serotype O157:H7 and its virulence factors in a single reaction. Analysis of 82 *E. coli* strains (49 O157:H7 and 33 non-O157:H7) demonstrated that this PCR system successfully distinguished serotype O157:H7 from other serotypes of *E. coli* and provided accurate profiling of the shiga-like toxins and the *intimin*** adhesin in individual strains. This multiplex PCR assay did not cross-react with the background bacterial flora in bovine faeces and could detect a single O157:H7 organism per gram of faeces when combined with an enrichment step. Together, these results indicate that the multiplex PCR assay can be used for specific identification and profiling of *E. coli* O157:H7 isolates, and may be applied to rapid and sensitive detection of *E. coli* O157:H7 in bovine faeces when combined with an enrichment step.

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10/3,AB/2 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal
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13922117 PASCAL No.: 99-0103959

Prevalence and molecular typing of attaching and effacing *Escherichia coli* among calf populations in Belgium

CHINA B; PIRSON V; MAINIL J

University of Liege, Faculty of Veterinary Medicine, Laboratory of Bacteriology, Liege, Belgium

Journal: Veterinary microbiology : (Amsterdam), 1998, 63 (2-4) 249-259

Language: English

Attaching and effacing *Escherichia coli* are involved in diarrhea in 2 to 8-week old calves. The virulence factors of these bacteria include: (i) the secretion of proteins (i.e. EspB) involved in microvilli effacement. (ii) the production of the *intimin***, a 94 kDa outer membrane protein encoded by the *eaeA*** gene and involved in the intimate attachment of bacteria to epithelial cell and (iii) the production of verotoxins: VT 1 and/or VT2. We investigated the presence and the pathotype of these strains in several calf populations by colony hybridization or by genetic amplification. Using the colony hybridization method we showed first that only 5% of calves who died from diarrhea presented *EaeA***+ *E. coli* strains and secondly that 19% of healthy calves showed an asymptomatic carriage. However, using

Searcher : Shears 308-4994

09/189415

colony hybridization and genetic amplification, we identified *EaeA*** strains in 91% of calves living in farms with recurrent diarrhea problems. In 66% of the calves, there was a correlation between the presence of AEEC and diarrhea. At the pathotype level, most of the *EaeA*** isolates were negative for VT probes. In VT+ bacteria, the majority were VT1+. The number of VT positive bacteria was significantly higher in calves who died from diarrhea than in healthy or sick calves. This underlined the aggravating role of verotoxins in the disease. Moreover, only 25% of the bovine AEEC were positive with the EaeB probe. Surprisingly, the proportion of EaeB+ strains was significantly higher in healthy calves than in other populations.

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10/3,AB/3 (Item 3 from file: 144)
DIALOG(R)File 144:Pascal
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12757078 PASCAL No.: 96-0470749
Typing of bovine attaching and effacing Escherichia coli by multiplex in vitro amplification of virulence-associated genes
CHINA B; PIRSON V; MAINIL J
Laboratory of Bacteriology, Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Belgium
Journal: Applied and environmental microbiology, 1996, 62 (9) 3462-3465
Language: English
Attaching and effacing Escherichia coli is a new causal agent of diarrhea in calves. Its major virulence factors are the *intimin*** protein, encoded by the *eaeA*** gene, and the Shiga-like toxins, encoded by sit genes. Because the sequences of these genes are available, we selected specific primers to amplify each virulence gene so as to develop a new identification test based on multiplex amplification of virulence-associated genes. Of 30 tested strains, 14 were *eaeA*** SUP + , 15 were *eaeA*** SUP + slt-I SUP + , 1 was *eaeA*** SUP + slt-I SUP + slt-II SUP + , and 1 was *eaeA*** SUP + slt-II SUP + . The method proved in our hands to be fast and specific and in perfect correlation with the hybridization method.

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10/3,AB/4 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP
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00310411
IDENTIFYING NO.: 1K08DK02864-01 AGENCY CODE: CRISP
PATHOGENESIS & INTERVENTION STRATEGIES IN HEMORRHAGIC COLITIS
Searcher : Shears 308-4994

09/189415

PRINCIPAL INVESTIGATOR: BLOOM, PETER D

ADDRESS: EMORY UNIVERSITY 1639 PIERCE DRIVE ATLANTA, GA 30322

PERFORMING ORG.: EMORY UNIVERSITY, ATLANTA, GEORGIA

SPONSORING ORG.: NAT INST OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

FY : 1999

SUMMARY: Candidate and Environment: The candidate, Dr. Bloom completed his G.I. fellowship and is now a junior faculty member of the G.I. Division at the University of Maryland. With the support of an NRSA, he studied attenuated Shigella vaccine constructs as vectors for foreign antigen delivery in an animal model. This work provided basic experience in molecular genetics. The candidate now seeks to become expert in these techniques under the supervision of Dr. James B. Kaper of the Center for Vaccine Development (CVD), the co-sponsor of this proposal and an authority in microbial molecular genetics. Because of his interest in hemolytic uremic syndrome (HUS) stemming from his documentation of an outbreak of Shigella dysenteriae in Southern Africa, Dr. Bloom will focus his attention on an animal model of hemorrhagic colitis developed by the co-sponsor, Dr. Edgar C. Boedeker of the G.I. Division and CVD. UNDER Drs. Boedeker and Kaper, the candidate hopes to acquire the molecular tools to investigate the pathogenesis of hemorrhagic colitis due to Shiga toxin producing E. coli (STEC) and to further develop his career as an independent investigator in the outstanding research environment of the CVD. Research: The broad aim of this proposal is to use a new animal model of STEC infection to understand the molecular pathogenesis of this disease. This approach should aid in the development of therapeutic regimens to prevent and treat STEC disease. Over the past decade STEC have emerged as important "pathogens", causing life threatening food-borne illness with numerous reports of hemorrhagic colitis, often complicated by the HUS, occurring in sporadic and epidemic outbreaks throughout the world. STEC produce potent protein toxins named Shiga toxins (Stx). In addition to Stx production, STEC share the ability to adhere intimately to intestinal epithelial cells by "attaching and effacing" (A/E) mechanisms. The most severe intestinal and renal manifestations of STEC infection result from toxin-mediated damage to microvascular endothelium, with tissue edema, inflammatory infiltrates, cytokine production, and vascular thrombi. Endotoxin and pro-inflammatory cytokines up-regulate Stx mediated tissue injury in vitro, but these effects have not been studied in vivo. Furthermore, A/E adherence of bacteria to intestinal epithelial cells, which is encoded for in the genetic locus of enterocyte effacement (LEE) may have a profound influence on the effectively delivery of toxin to the host. Specific aims of the proposal are to use an animal model of STEC infection to: 1. Examine the influence of A/E adherence on Stx toxicity by: a. producing a deletion mutation in *Tir* (the *translocated* *intimin* receptor) a critical virulence gene of the LEE, in the toxin-producing strain RDEC-H19A; b. using the products of the genes of the LEE, *intimin* and *TIR*, to actively immunize against STEC using a vaccine vector system developed for use in the rabbit model by the candidate. 2. Examine the sequential steps in the initiation and maintenance of inflammation, and the induction of vascular injury, by

Searcher : Shears 308-4994

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utilizing a selected group of antagonists in the in vivo model including:
a. the cytokine IL-11 which has effects on the maintenance of intestinal epithelial barrier function as well as inhibitory effects on macrophage activation; b. intraluminal and systemic endotoxin antagonists: i. Neutrophil BPI (bactericidal/permeability increasing); and ii. Limulus ENP (endotoxin neutralizing protein); c. anti IL-8 antibody to study the acute inflammatory effects of this chemokine; d. platelet activating factor (PAF) antagonists which affect platelet aggregation and acute inflammation.

10/3,AB/5 (Item 1 from file: 348)
DIALOG(R) File 348:European Patents
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00985690

ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Clostridium perfringens vaccine
Clostridium perfringens Impfstoff
Vaccine contre clostridium perfringens

PATENT ASSIGNEE:

Akzo Nobel N.V., (200754), Velperweg 76, 6824 BM Arnhem, (NL),
(applicant designated states:
AT;BE;CH;CY;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)

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LEGAL REPRESENTATIVE:

Ogilvie-Emanuelson, Claudia Maria et al (80441), Patent Department Pharma
N.V. Organon P.O. Box 20, 5340 BH Oss, (NL)
PATENT (CC, No, Kind, Date): EP 892054 A1 990120 (Basic)
APPLICATION (CC, No, Date): EP 98202032 980617;
PRIORITY (CC, No, Date): EP 97201888 970620
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;
LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; A61K-039/08; C07K-014/33;
C12N-001/21;

ABSTRACT EP 892054 A1

The present invention relates to detoxified immunogenic derivatives of Clostridium perfringens (beta)-toxin or an immunogenic fragment thereof that have as a characteristic that they carry a mutation in the (beta)-toxin amino acid sequence, not found in the wild-type (beta)-toxin amino acid sequence. The invention also relates to genes encoding such (beta)-toxins, as well as to expression systems expressing such

Searcher : Shears 308-4994

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(beta)-toxins. Moreover, the invention relates to bacterial expression systems expressing a native (beta)-toxin. Finally, the invention relates to vaccines based upon detoxified immunogenic derivatives of *Clostridium perfringens* (beta)-toxin, and methods for the preparation of such vaccines.

ABSTRACT WORD COUNT: 96

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	9903	583
SPEC A	(English)	9903	7428
Total word count - document A			8011
Total word count - document B			0
Total word count - documents A + B			8011

? ds; d 22/3,ab/1-12

Set	Items	Description
S11	661	AU=(FINLAY, B? OR FINLAY B?)
S12	250	AU=(KENNY, B? OR KENNY B?)
S13	24	AU=(DEVINNEY, R? OR DE VINNEY, R? OR DEVINNEY R? OR DE VIN- NEY R?)
S14	2922	AU=(STEIN, M? OR STEIN M?)
S15	3	S11 AND S12 AND S13 AND S14
S16	58	S11 AND (S12 OR S13 OR S14)
S17	17	S12 AND (S13 OR S14)
S18	6	S13 AND S14
S19	3776	S11 OR S12 OR S13 OR S14
S20	24	(S16 OR S19) AND S1
S21	24	(S15 OR S17 OR S18 OR S20) NOT (S5 OR S9)
S22	12	RD (unique items)

- Author(s)

? t 22/3,ab/1-12

>>>No matching display code(s) found in file(s): 65, 113

22/3,AB/1 (Item 1 from file: 65)
DIALOG(R)File 65:Inside Conferences
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02302103 INSIDE CONFERENCE ITEM ID: CN024112431

Molecular mechanisms of enteropathogenic *E. coli*: Signal transduction, pedestal formation, intimate contact, and diarrhea

Finlay, B. B.; *Kenny, B."**"; *Stein, M."**"; Reinscheid, D.

CONFERENCE: Enteropathogenic *Escherichia coli*-International symposium

REVISTA DE MICROBIOLOGIA, 1996; VOL 27; SUPP 1 P: 95-98

(np), 1996

ISSN: 0001-3714

LANGUAGE: English DOCUMENT TYPE: Conference Papers

CONFERENCE EDITOR(S): Kaper, J. B.

Searcher : Shears 308-4994

09/189415

CONFERENCE LOCATION: Sao Paulo, Brazil
CONFERENCE DATE: Aug 1995 (199508) (199508)

22/3,AB/2 (Item 2 from file: 65)
DIALOG(R)File 65:Inside Conferences
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01741681 INSIDE CONFERENCE ITEM ID: CN017738833
Enteropathogenic E. coli Exploitation of Host Epithelial Cells
Finlay, B. B.; Ruschkowski, S.; *Kenny, B."**; *Stein, M."**
CONFERENCE: Microbial pathogenesis and immune response-Meeting; 2nd
ANNALS- NEW YORK ACADEMY OF SCIENCES, 1996; VOL 797 P: 26-31
New York Academy of Sciences, 1996
ISSN: 0077-8923 ISBN: 1573310166; 1573310174
LANGUAGE: English DOCUMENT TYPE: Conference Papers
CONFERENCE EDITOR(S): Ades, E. W.; Morse, S. A.; Rest, R. F.
CONFERENCE LOCATION: New York, NY
CONFERENCE DATE: Oct 1995 (199510) (199510)

22/3,AB/3 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal
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14307127 PASCAL No.: 99-0513847
Type III secretion-dependent hemolytic activity of enteropathogenic
Escherichia coli
WARAWA J; *FINLAY B B"**; *KENNY B"**
Department of Pathology and Microbiology, School of Medical Sciences,
Bristol, United Kingdom; Biotechnology Laboratory, Vancouver, British
Columbia, V6T 1Z3, Canada
Journal: Infection and immunity, 1999, 67 (10) 5538-5540
Language: English
Enteropathogenic Escherichia coli (EPEC) was found to exhibit a type III
secretion-dependent, contact-mediated, hemolytic activity requiring the
EspA, EspB, and EspD secreted proteins. EspB and EspD display homology to
pore-forming molecules. Our data suggest a mechanism to explain the
requirement for all three Esp proteins in the transfer of EPEC proteins,
such as *Tir"**, into target cells.

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22/3,AB/4 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal
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14080585 PASCAL No.: 99-0273444
Searcher : Shears 308-4994

09/189415

Enteropathogenic Escherichia coli : cellular harassment

Host-microbe interactions: bacteria

*DEVINNEY R***; KNOECHEL D G; *FINLAY B B***

COSSART Pascale, ed; MILLER Jeff F, ed

Biotechnology Laboratory, University of British Columbia, Vancouver,
British Columbia, V6T 1Z4, Canada

Unite des Interactions Bacteries-Cellules, Institut Pasteur, 28 rue du Dr
Roux, 75015 Paris, France; University of California Los Angeles School of
Medicine, Dept of Microbiology and Immunology, 10833 Le Conte Ave., Los
Angeles, CA 90024, United States

Journal: Current opinion in microbiology, 1999, 2 (1) 83-88

Language: English

The mechanisms by which enteropathogenic Escherichia coli (EPEC) mediates
diarrhea remain a mystery. Recently a number of interesting and at times
surprising results have come from studying EPEC interactions with host
cells. Identification and characterization of bacterial factors, including
*Tir***, EspA, EspB and EspD, and host responses have expanded our grasp of
the diverse effects of EPEC on host cells.

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22/3,AB/5 (Item 3 from file: 144)

DIALOG(R)File 144:Pascal

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13217033 PASCAL No.: 97-0484125

Characterization of two virulence proteins secreted by rabbit
enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression
is sensitive to host body temperature

ABE A; *KENNY B***; *STEIN M***; FINLAY B B

Biotechnology, Laboratory, University of British Columbia, Vancouver,
British Columbia, V6T 1Z3, Canada; Department of Bacteriology, The Kitasato
Institute, Minato-ku, Tokyo 108, Japan

Journal: Infection and immunity, 1997, 65 (9) 3547-3555

Language: English

Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause
unique histopathological features on intestinal mucosa, including
attaching/effacing (A/E) lesions. Due to the human specificity of EPEC,
RDEC-I has been used as an animal model to study EPEC pathogenesis. At
least two of the previously identified EPEC-secreted proteins, EspA and
EspB, are required for triggering host epithelial signal transduction
pathways, intimate adherence, and A/E lesions. However, the functions of
these secreted proteins and their roles in pathogenesis have not been
characterized. To investigate the function of EspA and EspB in RDEC-1, the
espA and espB genes were cloned and their sequences were compared to that
of EPEC 0127. The EspA proteins showed high similarity (88.5% identity),
while EspB was heterogeneous in internal regions (69.8% identity). However,
RDEC-I EspB was identical to that of enterohemorrhagic E. coli serotype

Searcher : Shears 308-4994

026. Mutations in RDEC-I espA and espB revealed that the corresponding RDEC-I gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing EPEC espA or/and espB genes into RDEC-I mutant strains demonstrated that they were functionally interchangeable, although the EPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.

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22/3,AB/6 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal
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13078049 PASCAL No.: 97-0369601

Enteropathogenic Escherichia coli protein secretion is induced in response to conditions similar to those in the gastrointestinal tract

*KENNY B***; ABE A; *STEIN M***; FINLAY B B

Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T-1Z3, Canada

Journal: Infection and immunity, 1997, 65 (7) 2606-2612

Language: English

The pathogenicity of enteropathogenic Escherichia coli (EPEC) is associated with the expression and secretion of specific bacterial factors. EspB is one such secreted protein which is required to trigger host signaling pathways resulting in effacement of microvilli and cytoskeletal rearrangements. These events presumably contribute to the ensuing diarrhea associated with EPEC infections. EPEC encounters several environmental changes and stimuli during its passage from the external environment into the host gastrointestinal tract. In this paper we show that the secretion of EspB is subject to environmental regulation, and maximal secretion occurs under conditions reminiscent of those in the gastrointestinal tract. Thus, secretion is maximal at 37 Degree C, pH 7, and physiological osmolarity. In addition, maximal secretion requires the presence of sodium bicarbonate and calcium and is stimulated by millimolar concentrations of Fe(NO SUB 3) SUB 3 . The secretion of the four other EPEC-secreted proteins appears to be modulated in a manner similar to that of EspB. Our results also show that secretion is not dependent on CO SUB z , as originally reported by Haigh et al. (FEMS Microbiol. Lett. 129: 63-67, 1995), but that CO SUB z more likely acts as a component of the medium buffering system, since CO SUB 2 dependence was abolished by the use of alternative buffers.

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22/3,AB/7 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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08760754 GENUINE ARTICLE#: XT420 NUMBER OF REFERENCES: 40
TITLE: Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature
AUTHOR(S): Abe P; *Kenny B***; *Stein M***; Finlay BB (REPRINT)
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB, ROOM 237, WESBROOK BLDG, 6174 UNIV BLVD/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; KITASATO INST,DEPT BACTERIOL, MINATO KU/TOKYO 108//JAPAN/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N9 (SEP), P3547-3555
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-1 has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified FPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC O127, The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-1 EspB was identical to that of enterohemorrhagic E. coli serotype O26. Mutations in RDEC-1 espA and espB revealed that the corresponding RDEC-1 gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing FPEC espA or/and espB genes into RDEC-1 mutant strains demonstrated that they were functionally interchangeable, although the FPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.
ISSN: 0019-9567

22/3,AB/8 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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Searcher : Shears 308-4994

09/189415

07901289 GENUINE ARTICLE#: VR931 NUMBER OF REFERENCES: 56

TITLE: Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic *Escherichia coli* which is homologous to members of the immunoglobulinA protease-like family of secreted proteins

AUTHOR(S): *Stein M***; *Kenny B***; *Stein MA***; Finlay BB

CORPORATE SOURCE: UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA, DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/

PUBLICATION TYPE: JOURNAL

PUBLICATION: JOURNAL OF BACTERIOLOGY, 1996, V178, N22 (NOV), P6546-6554

PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171

ISSN: 0021-9193

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Enteropathogenic *Escherichia coli* (EPEC) secretes at least five proteins. Two of these proteins, EspA and EspB (previously called EaeB), activate signal transduction pathways in host epithelial cells. While the role of the other three proteins (39, 40, and 110 kDa) remains undetermined, secretion of all five proteins is under the control of pcrA, a known positive regulator of several EPEC virulence factors. On the basis of amino-terminal protein sequence data, we cloned and sequenced the gene which encodes the 110-kDa secreted protein and examined its possible role in EPEC signaling and interaction with epithelial cells. In accordance with the terminology used for cspA, and espB, we called this gene espC, for EPEC-secreted protein C. We found significant homology between the predicted EspC protein sequence and a family of immunoglobulin A (IgA) protease-like proteins which are widespread among pathogenic bacteria. Members of this protein family are found in avian pathogenic *Escherichia coli* (Tsh), *Haemophilus influenzae* (Hap), and *Shigella flexneri* (SepA). Although these proteins and EspC do not encode IgA protease activity, they have considerable homology with IgA protease from *Neisseria gonorrhoeae* and *H. influenzae* and appear to use a export system for secretion. We found that genes homologous to espC also exist in other pathogenic bacteria which cause attaching and effacing lesions, including *Hafnia alvei* biotype 19982, *Citrobacter freundii* biotype 4280, and rabbit diarrheagenic *E. coli* (RDEC-1). Although these strains secrete various proteins similar in molecular size to the proteins secreted by EPEC, we did not detect secretion of a 110-kDa protein by these strains. To examine the possible role of EspC in EPEC interactions with epithelial cells, we constructed a deletion mutant in espC by allelic exchange and characterized the mutant by standard tissue culture assays. We found that EspC is not necessary for mediating EPEC-induced signal transduction in HeLa epithelial cells and does not play a role in adherence or invasion of tissue culture cells.

ISSN: 0021-9193

Searcher : Shears 308-4994

09/189415

22/3,AB/9 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents
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01088531

ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
METHODS FOR ASSAYING TYPE III SECRETION INHIBITORS
PROCEDES D'ANALYSE D'INHIBITEURS DE SECRETION DE TYPE III
PATENT ASSIGNEE:

UNIVERSITY OF BRITISH COLUMBIA, (917321), Room 331, IRC Building, 2194
Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3, (CA),
(Applicant designated States: all)

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PATENT (CC, No, Kind, Date):

WO 9945136 990910

APPLICATION (CC, No, Date): WO 99937945 990305; WO 99CA183 990305

PRIORITY (CC, No, Date): US 76980 P 980305

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;
LU; MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: C12Q-001/02; C12Q-001/32; C12Q-001/34;
C12Q-001/42; C12Q-001/48; C12Q-001/66; G01N-033/68

LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/10 (Item 2 from file: 348)
DIALOG(R)File 348:European Patents
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PATHOGENIC ESCHERICHIA COLI ASSOCIATED PROTEIN
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PROTEINE ASSOCIEE A UN ESCHERICHIA COLI PATHOGENE
PATENT ASSIGNEE:

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designated states:
AT;BE;CH;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)

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Searcher : Shears 308-4994

09/189415

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PATENT (CC, No, Kind, Date): EP 904288 A2 990331 (Basic)

WO 9740063 971030

APPLICATION (CC, No, Date): EP 97917185 970423; WO 97CA265

PRIORITY (CC, No, Date): US 15999 P 960423

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU;
MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: C07K-014/00

NOTE:

No A-document published by EPO

LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/11 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotechnology Abs

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0243573 DBA Accession No.: 1999-14338 PATENT

Identifying antibacterial agents that inhibit Gram-negative type-III
secretion system, for treating infections -by screening for inhibition
of virulence factors secreted by this system - e.g. plasmid
pMS21-mediated EspB gene, herpes simplex virus tag gene transfer and
expression in Escherichia coli

AUTHOR: Finlay B B; *Kenny B***; *Stein M***

CORPORATE SOURCE: Vancouver, British Columbia, Canada.

PATENT ASSIGNEE: Univ.British-Columbia 1999

PATENT NUMBER: WO 9945136 PATENT DATE: 19990910 WPI ACCESSION NO.:

1999-540860 (1945)

PRIORITY APPLIC. NO.: US 76980 APPLIC. DATE: 19980305

NATIONAL APPLIC. NO.: WO 99CA183 APPLIC. DATE: 19990305

LANGUAGE: English

ABSTRACT: Identification of antibacterial agents is new and involves
treating bacteria that contain a polynucleotide which encodes a protein
secreted by the type-III secretion system (3SS) with a test compound
and detecting secretion of the protein. A reduction of secretion,
relative to that in bacteria not treated with the test compound,
indicates an inhibitor of 3SS. Also claimed is a kit containing in
separate containers, the bacteria and a system for detecting secretion
of the protein. The antibacterial agents can be used to treat
infections in humans other animals and plants, e.g. where caused by
enteropathogenic or enterohemorrhagic Escherichia coli, Yershi sp.,
Shigella sp., Pseudomonas aeruginosa, Pseudomonas syringae, Xanthomonas
campestris or many others, for analyzing the functional mechanisms of
3SS and for development of more powerful or specific inhibitors. In an
example, plasmid pMS21 containing a sequence encoding the N-terminal
part of protein EspB and a sequence encoding a herpes simplex virus tag
against which commercial antibiotics are available was used to

Searcher : Shears 308-4994

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S3	3067	(S1 OR S2) AND (PROTEIN? ? OR POLYPROTEIN? ? OR POLYPEPTID- E? ? OR PEPTIDE? ?)
S4	114	S3 AND (EPEC OR EHEC OR (ENTEROHEMORRH? OR ENTEROHAEMORRH? OR ENTEROPATHOGEN? OR ENTERO(W) (HEMORRH? OR HAEMORRH? OR PATH- OGEN?)) (5N) COLI OR "A/E" OR ATTACH? (1W) EFFAC?)
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S7	37	RD (unique items)

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7/3,AB/1 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal
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15072746 PASCAL No.: 01-0232088
Human response to Escherichia coli O157:H7 infection : Antibodies to

Searcher : Shears 308-4994

-key terms

secreted virulence factors

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Hospital Civic Campus, Ottawa, Ontario, K1Y 4E9, Canada

Journal: Infection and immunity, 2000, 68 (9) 5090-5095

Language: English

Vaccination has been proposed for the prevention of disease due to *enterohemorrhagic*** Escherichia coli*** (*EHEC***), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:H7, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different *EHEC*** virulence factors: *Tir*** (*translocated*** *intimin*** *receptor***, which is inserted into the host cell membrane), *intimin*** (bacterial outer membrane *protein*** which *binds*** to *Tir***), EspA (secreted *protein*** which forms filamentous structures on *EHEC*** surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examined. Sera were assayed against purified recombinant *proteins*** using immunoblot analysis and by enzyme-linked immunosorbent assay to determine the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA, EspB, and *intimin*** in the acute-phase sera, although there was some reactivity to *Tir***. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against *Tir*** (up to a titer of 1:256,000), especially in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for *Tir***. These results suggest that there is a strong immune response to *Tir***, and to a lesser extent to the other three virulence factors, following *EHEC*** disease, indicating that these bacterial molecules are potential vaccine candidates for preventing *EHEC*** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (*Tir*** or EspB) are still recognized by the host immune response.

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7/3,AB/2 (Item 2 from file: 144)

DIALOG(R) File 144:Pascal

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15044716 PASCAL No.: 01-0202245

*Intimin*** from Shiga toxin-producing Escherichia coli and its isolated C-terminal domain exhibit different *binding*** properties for *Tir*** and a eukaryotic surface receptor

09/189415

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Journal: International journal of medical microbiology, 2001, 290 (8)
683-691

Language: English

The outer membrane *protein*** *intimin*** plays a crucial role in the *attaching*** and *effacing*** process employed by different enteropathogens to colonize the epithelial surface of their hosts. In this study we have characterized the C-terminal *binding*** domain of *intimin*** from the Shiga toxin-producing Escherichia coli strain 413/89-1, that belongs to the beta -subtype of intimins. We found that a fusion of this domain to the maltose-*binding*** *protein*** *binds*** efficiently to both the *translocated*** *intimin*** *receptor*** (*Tir***) and the surface of uninfected eukaryotic host cells. In contrast, no such *binding*** was observed with the full-length *protein*** localized on the bacterial surface. As the C-terminal domain of *intimin*** and the full-length *protein*** differ in their *binding*** activity, we suggest that the intiminbinding domain might be controlled by the N-terminal portion of the molecule to prevent unproductive interactions with molecules in the lumen of the gut.

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7/3,AB/3 (Item 3 from file: 144)

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14970659 PASCAL No.: 01-0123867

Mechanical fractionation reveals structural requirements for *enteropathogenic*** Escherichia *coli*** *Tir*** insertion into host membranes

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Journal: Infection and immunity, 2000, 68 (7) 4344-4348

Language: English

*Enteropathogenic*** Escherichia *coli*** (*EPEC***) inserts its receptor for intimate adherence (*Tir***) into host cell membranes by using a type III secretion system. Detergents are frequently used to fractionate infected host cells to investigate bacterial *protein*** delivery into mammalian cells. In this study, we found that the Triton X-100-soluble membrane fraction from *EPEC***-infected HeLa cells was contaminated with bacterial *proteins***. We therefore applied a mechanical method of cell lysis and ultracentrifugation to fractionate infected HeLa cells to

investigate the biology and biochemistry of *Tir*** delivery and translocation. This method demonstrates that the translocation of *Tir*** into the host cell membrane requires its transmembrane domains, but not tyrosine phosphorylation or *binding*** to *Tir***'s ligand, *intimin***.

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7/3,AB/4 (Item 4 from file: 144)
DIALOG(R) File 144:Pascal
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14713466 PASCAL No.: 00-0388975

*Intimin*** from *enteropathogenic*** Escherichia coli*** mediates remodelling of the eukaryotic cell surface

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Journal: Microbiology : (Reading), 2000, 146 (p.6) 1333-1344

Language: English

Adhesion to cultured epithelial cells by *enteropathogenic*** Escherichia coli*** (*EPEC***) is associated with extensive rearrangement of the host cell cytoskeleton. Evidence has been presented that *EPEC*** adhesion is associated with activation of signal transduction pathways leading to production of a characteristic histopathological feature known as the *attaching*** and *effacing*** (A/E) lesion. A/E lesion formation requires *intimin***, an *EPEC*** adhesion molecule and several *EPEC*** secreted *proteins*** (EspA, B, D and *Tir***) involved in cell signalling and *protein*** translocation. In this study it is shown that HEP-2 cells respond during the early stages of infection with two wild-type *EPEC*** strains (B171 and E2348/69) by producing microvillus-like processes (MLP) at the site of initial bacterial adherence. *Intimin*** appears to play a key role in MLP elongation. At later stages of infection with these wild-type *EPEC*** strains, when A/E lesions have formed, the MLP were reduced in number and length to appear as at time zero, and the cell surface in the vicinity of bacterial clusters appeared unaffected. In contrast, infection with EspA- or EspB-negative, but *intimin***-positive, *EPEC*** strains (UMD872 and UMD864, respectively) resulted in enhanced MLP proliferation and formation of 'cage-like' structures engulfing the bacteria. Inoculating HEP-2 cells with *intimin***-coated latex spheres induced similar 'cage-like' structures. Caco-2 cells did not show *intimin***-induced microvillus elongation in response to *EPEC*** infection, although microvillus effacement and reduction in number occurred. Similar phenomena appeared on B171 and E2348/69 infection of paediatric intestine using in vitro organ culture, i.e. elongated

microvilli were seen in association with small colonies and at the periphery of large localized colonies, along with evidence of microvillus breakdown and debris in the colony centre. These results show that *intimin*** activates signal transduction pathways involved in the remodelling of the eukaryotic cell surface, probably via *binding*** to a receptor encoded by the host cell.

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7/3,AB/5 (Item 5 from file: 144)
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14660969 PASCAL No.: 00-0333697

Crystal structure of *enteropathogenic*** Escherichia *coli***
 *intimin***-receptor complex

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 FINLAY B B; STRYNADKA N C J

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Journal: Nature : (London), 2000, 405 (6790) 1073-1077

Language: English

*Intimin*** and its *translocated*** *intimin*** *receptor*** (*Tir***) are bacterial *proteins*** that mediate adhesion between mammalian cells and *attaching*** and *effacing*** (A/E) pathogens. *Enteropathogenic*** Escherichia *coli*** (*EPEC***) causes significant paediatric morbidity and mortality world-wide SUP 1 , A related A/E pathogen, *enterohaemorrhagic*** E. *coli*** (*EHEC***; O157:H7) is one of the most important food-borne pathogens in North America, Europe and Japan. A unique and essential feature of A/E bacterial pathogens is the formation of actin-rich pedestals beneath the intimately adherent bacteria and localized destruction of the intestinal brush border SUP 2 . The bacterial outer membrane adhesin, *intimin*** SUP 3 , is necessary for the production of the A/E lesion and diarrhoea SUP 4 . The A/E bacteria translocate their own receptor for *intimin***, *Tir*** SUP 5 , into the membrane of mammalian cells using the type III secretion system. The translocated *Tir*** triggers additional host signalling events and actin nucleation, which are essential for lesion formation. Here we describe the the crystal structures of an *EPEC*** *intimin*** carboxy-terminal fragment alone and in complex with the *EPEC*** *Tir*** *intimin***-binding*** domain, giving insight into the molecular mechanisms of adhesion of A/E pathogens.

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7/3,AB/6 (Item 6 from file: 144)

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14409823 PASCAL No.: 00-0065744

Human colostrum and serum contain antibodies reactive to the *intimin***-
*binding*** region of the *enteropathogenic*** Escherichia *coli***
*translocated*** *intimin*** *receptor***

IMPERIO SANCHES M; KELLER R; HARTLAND E L; FIGUEIREDO D M M; BATCHELOR M;
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Journal: Journal of pediatric gastroenterology and nutrition, 2000, 30 (1)
73-77

Language: English

Background: In Brazil, *enteropathogenic*** Escherichia *coli*** (*EPEC***) diarrhoea is endemic in young infants. A characteristic feature of *EPEC*** adhesion to host cells is intimate attachment leading to the formation of distinctive "*attaching*** and *effacing***" (A/E) lesions on mammalian cells. Two genes directly involved in intimate adhesion, eae and *tir***, encode the adhesion molecule *intimin*** and its translocated receptor *Tir***, respectively. The *intimin***-*binding*** domain of *Tir*** was recently mapped to the middle part of the *polypeptide*** (*Tir***-M), and the amino (*Tir***-N) and carboxy (*Tir***-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of *proteins*** associated with *EPEC*** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli 0157 can produce antibodies to *Tir***. In the current study antibody responses to the different *Tir*** domains were analyzed in sera and colostrum samples collected in an *EPEC***-endemic area of Brazil. Methods: Recombinant *Tir***, *Tir***-N, *Tir***-M, and *Tir***-C were expressed as His-tagged *protein*** in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the *Tir*** fragments. Results: Anti-*Tir*** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea. Anti-*Tir*** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the *Tir***-*polypeptide***, *Tir*** M, was identified. Conclusion: The *intimin***-*binding*** region of *Tir*** (*Tir***-M) is the immunodominant region of the *polypeptide*** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the *Tir***-M domain.

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7/3,AB/7 (Item 7 from file: 144)
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14116878 PASCAL No.: 99-0312625

*Enterohemorrhagic*** Escherichia *coli*** O157:H7 produces *Tir***, which is translocated to the host cell membrane but is not tyrosine phosphorylated

DEVINNEY R; STEIN M; REINSCHIED D; ABE A; RUSCHKOWSKI S; FINLAY B B
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Journal: Infection and immunity, 1999, 67 (5) 2389-2398

Language: English

Intimate attachment to the host cell leading to the formation of *attaching*** and *effacing*** (A/E) lesions is an essential feature of *enterohemorrhagic*** Escherichia *coli*** (*EHEC***) O157:H7 pathogenesis. In a related pathogen, *enteropathogenic*** E. *coli*** (*EPEC***), this activity is dependent upon translocation of the *intimin*** receptor, *Tir***, which becomes tyrosine phosphorylated within the host cell membrane. In contrast, the accumulation of tyrosine-phosphorylated *proteins*** beneath adherent *EHEC*** bacteria does not occur, leading to questions about whether *EHEC*** uses a *Tir***-based mechanism for adherence and A/E lesion formation. In this report, we demonstrate that *EHEC*** produces a functional *Tir*** that is inserted into host cell membranes, where it serves as an *intimin*** receptor. However, unlike in *EPEC***, in *EHEC*** *Tir*** is not tyrosine phosphorylated yet plays a key role in both bacterial adherence to epithelial cells and pedestal formation. *EHEC***, but not *EPEC***, was unable to synthesize *Tir*** in Luria-Bertani medium but was able to secrete *Tir*** into M9 medium, suggesting that *Tir*** synthesis and secretion may be regulated differently in these two pathogens. *EHEC*** *Tir*** and *EPEC*** *Tir*** both *bind*** *intimin*** and focus cytoskeletal rearrangements, indicating that tyrosine phosphorylation is not needed for pedestal formation. *EHEC*** and *EPEC*** intimins are functionally interchangeable, but *EHEC*** *Tir*** shows a much greater affinity for *EHEC*** *intimin*** than for *EPEC*** *intimin***. These findings highlight some of the differences and similarities between *EHEC*** and *EPEC*** virulence mechanisms, which can be exploited to further define the molecular basis of pedestal formation.

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7/3,AB/8 (Item 8 from file: 144)
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09/189415

13999988 PASCAL No.: 99-0184953

*Enteropathogenic*** Escherichia coli*** inhibits phagocytosis

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Vancouver, British Columbia V6T 1Z3, Canada

Journal: Infection and immunity, 1999, 67 (2) 490-495

Language: English

*Enteropathogenic*** Escherichia coli*** (*EPEC***) interacts with
intestinal epithelial cells, activating host signaling pathways leading to
cytoskeletal rearrangements and ultimately diarrhea. In this study, we
demonstrate that *EPEC*** interacts with the macrophage-like cell line
J774A.1 to inhibit phagocytosis by these cells. Antiphagocytic activity was
also observed in cultured RAW macrophage-like cells upon *EPEC***
infection. The *EPEC*** antiphagocytic phenotype was dependent on the type
III secretion pathway of *EPEC*** and its secreted *proteins***, including
EspA, EspB, and EspD. *Intimin*** and *Tir*** mutants displayed
intermediate antiphagocytic activity, suggesting that intimate attachment
mediated by *intimin***-*Tir*** *binding*** may also play a role in
antiphagocytosis. Tyrosine dephosphorylation of several host *proteins***
was observed following infection with secretion-competent *EPEC*** but not
with secretion-deficient mutants. Dephosphorylation was detectable 120 min
after infection with *EPEC***, directly correlating with the onset of the
antiphagocytic phenotype. Inhibition of *protein*** tyrosine phosphatases
by pervanadate treatment increased the number of intracellular wild-type
*EPEC*** organisms to levels seen with secretion-deficient mutants,
suggesting that dephosphorylation events are linked to the antiphagocytic
phenotype. No tyrosine phosphatase activity was detected with the *EPEC***
-secreted *proteins***, suggesting that *EPEC*** induces antiphagocytosis
via a different mechanism than Yersinia species. Taken together, the
present findings demonstrate a novel function for *EPEC***-secreted
*proteins*** in triggering macrophage *protein*** tyrosine
dephosphorylation and inhibition of phagocytosis.

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7/3,AB/9 (Item 9 from file: 144)

DIALOG(R)File 144:Pascal

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13868156 PASCAL No.: 99-0046090

*Translocated*** *intimin*** *receptors*** (*Tir***) of shiga-toxigenic
Escherichia coli isolates belonging to serogroups O26, O111, and O157 react
with sera from patients with hemolytic-uremic syndrome and exhibit marked
sequence heterogeneity

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Journal: Infection and immunity, 1998, 66 (11) 5580-5586

Language: English

The capacity to form *attaching*** and *effacing*** (A/E) lesions on the surfaces of enterocytes is an important virulence trait of several enteric pathogens, including *enteropathogenic*** Escherichia coli*** (*EPEC***), and Shiga-toxigenic E. coli (STEC). Formation of such lesions depends upon an interaction between a bacterial outer membrane *protein*** (*intimin***) and a bacterially encoded receptor *protein*** (*Tir***) which is exported from the bacterium and translocated into the host cell membrane. *Intimin***, *Tir***, and several other *proteins*** necessary for generation of A/E lesions are encoded on a chromosomal pathogenicity island termed the locus for enterocyte effacement (LEE). Reports of sequence heterogeneity and antigenic variation in the region of *intimin*** believed to be responsible for receptor *binding*** raise the possibility that the receptor itself is also heterogeneous. We have examined this by cloning and sequencing *tir*** genes from three different STEC strains belonging to serogroups O26, O111, and O157. The deduced amino acid sequences for the *Tir*** homologues from these strains varied markedly, exhibiting only 65.4, 80.2, and 56.7% identity, respectively, to that recently reported for *EPEC*** *Tir***. STEC *Tir*** is also highly immunogenic in humans. Western blots of E. coli DH5 alpha expressing the various STEC *tir*** genes cloned in pBluescript (but not E. coli DH5 alpha (pBluescript)) reacted strongly with convalescent sera from patients with hemolytic-uremic syndrome (HUS) caused by known LEE-positive STEC. Moreover, no reaction was seen when the various clone lysates were probed with serum from a patient with HUS caused by a LEE-negative STEC or with serum from a healthy individual. Covariation of exposed epitopes on both *intimin*** and *Tir*** may be a means whereby STEC avoid host immune responses without compromising adhesin-receptor interaction.

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7/3,AB/10 (Item 10 from file: 144)
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13078038 PASCAL No.: 97-0369590

*Intimin***-dependent *binding*** of *enteropathogenic*** Escherichia coli*** to host cells triggers novel signaling events, including tyrosine phosphorylation of phospholipase C- gamma 1

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British Columbia, V6T 1Z3, Canada

Journal: Infection and immunity, 1997, 65 (7) 2528-2536

Language: English

*Enteropathogenic*** Escherichia coli*** (*EPEC***). interactions with HeLa epithelial cells induced the tyrosine phosphorylation of a host *protein*** of approximately 150 kDa, Hp150. Phosphorylation of this *protein*** band was dependent on the interaction of the *EPEC*** *protein*** *intimin*** with epithelial cell surfaces and was correlated with pedestal formation. Hp150 phosphorylation was specifically inhibited by the addition of cytochalasin D, an inhibitor of actin polymerization, although this appeared to be an indirect effect preventing interaction of *intimin*** with its receptor, tyrosine-phosphorylated *Hp90***, and thus triggering Hp150 phosphorylation. This suggests the involvement of an actin-based movement of membrane-bound tyrosine-phosphorylated *Hp90*** to allow its interaction with *intimin***. Analysis of the tyrosine-phosphorylated Hp150 *protein*** demonstrated that it is heterogeneous in composition, with phospholipase C- gamma 1 (PLC- gamma 1) being a minor component. Activation of PLC- gamma 1 by tyrosine phosphorylation leads to inositol triphosphate and Ca SUP 2 SUP + fluxes, events detected following *EPEC*** infection. *EPEC*** also induced tyrosine dephosphorylation of host *proteins***, including a 240-kDa host *protein*** (Hp240), following *EPEC*** infection. *Protein*** dephosphorylation appears to be a signaling event which occurs independently of *intimin***. Inhibition of host tyrosine dephosphorylation events by the addition of the tyrosine phosphatase inhibitor sodium vanadate did not prevent actin accumulation beneath the adherent bacteria. We conclude that *EPEC*** induces two sets of signaling events following infection. One set is dependent on *EPEC*** *proteins*** secreted by the type III secretion pathway (EspA and EspB) which induces *Hp90*** tyrosine phosphorylation and dephosphorylation of host phosphotyrosine *proteins***. The second set, which is also dependent on the first signaling events, requires *intimin*** interaction with its receptor, tyrosine-phosphorylated *Hp90***, to trigger Hp150 and PLC- gamma 1 tyrosine phosphorylation as well as pedestal formation. Inhibition of pedestal formation by tyrosine kinase inhibitors indicates an important role for tyrosine phosphorylation events during *EPEC*** subversion of host processes.

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7/3,AB/11 (Item 11 from file: 144)
DIALOG(R)File 144:Pascal
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12483865 PASCAL No.: 96-0147695

Expression of *attaching***/*effacing*** activity by *enteropathogenic*** Escherichia coli*** depends on growth phase, temperature, and *protein*** synthesis upon contact with epithelial cells

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09/189415

Journal: Infection and immunity, 1996, 64 (3) 966-973

Language: English

*Enteropathogenic*** Escherichia coli*** (*EPEC***) induces tyrosine phosphorylation of a 90***-kDa*** protein*** (*Hp90***) in infected epithelial cells. This in turn facilitates intimate binding*** of *EPEC*** via the outer membrane protein*** intimin***, effacement of host cell microvilli, cytoskeletal rearrangement, and bacterial uptake. This phenotype has been commonly referred to as attaching***/effacing*** (A/E). The ability of *EPEC*** to induce A/E lesions was dependent on bacterial growth phase and temperature. Early-logarithmic-phase *EPEC*** grown at 37 Degree C elicits strong A/E activity within minutes after infection of HeLa epithelial cells. *EPEC*** de novo protein*** synthesis during the first minutes of interaction with the host cell was required to elicit A/E lesions. However, once formed, bacterial viability was not needed to maintain A/E lesions. The type of growth media and partial O SUB 2 pressure level do not seem to affect the ability of *EPEC*** to cause A/E lesions. These results indicates that the A/E activity of *EPEC*** is tightly regulated by environmental and host factors.

7/3,AB/12 (Item 1 from file: 266)

DIALOG(R)File 266:FEDRIP

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IDENTIFYING NO.: 5R01AI46454-02 AGENCY CODE: CRISP

HOST CELL SIGNALING BY *EHEC*** *INTIMIN*** *PROTEIN***

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SPONSORING ORG.: NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

FY : 2001

SUMMARY: *Enterohemorrhagic*** E. coli*** (*EHEC***) has emerged as an important agent of diarrheal disease and the leading cause of pediatric renal failure in the U.S. Intimate attachment to host cells is an essential step during intestinal colonization by EH EC. After initial host cell attachment, the bacterium injects into the host cell a number of molecules that trigger signaling pathways and result in the disruption of the eukaryotic cytoskeleton. Among the injected proteins*** is Tir***, a protein that becomes localized in the host cell membrane and acts as a receptor for the bacterial outer membrane protein*** intimin***. Intimin***, encoded by the eae gene, is required for the formation of a highly organized for the formation of a highly organized cytoskeletal structure containing filamentous actin directly beneath the bound bacterium that lifts the bacterium above the plane of the host cell membrane on a "pedestal". Deletion mutants of eae, which cannot induce the

Searcher : Shears 308-4994

formation of this pedestal, are deficient for intestinal colonization. Thus, we postulate that *Tir***-*intimin*** interaction is an essential early event in the development of disease caused by *EHEC***. We have identified regions of *intimin*** and *Tir*** that interact with each other, and have shown that the *Tir***-*binding*** region of *intimin*** is sufficient to induce actin condensation after pre-infection of host cells with *E. coli*. A detailed understanding of *Tir***-*intimin*** *binding***, as well as of the molecular signals immediately downstream of this interaction, are required to gain insight into how *EHEC*** colonizes the intestine and promotes damage. Thus, the following questions will be addressed: 1. What is the topological map of *Tir*** in the eukaryotic membrane? 2. Is *Tir*** *binding*** by *intimin*** sufficient to trigger actin condensation on preinfected cells? Latex beads that artificially *bind*** *TIR*** will be tested for the ability to induce actin condensation on preinfected eukaryotic cells. 3. How does *intimin*** and *Tir*** recognize each other? Genetic and biochemical approaches, including crystallographic studies, will be pursued to understand the molecular basis for this interaction. 4. Is *Tir***-*intimin*** interaction essential to promote intestinal colonization? Point mutations in *eae* and *tir*** that disrupt or restore *Tir***-*intimin*** *binding*** will be tested for their effect on colonization in an animal model for *EHEC*** infection. 5. What mammalian cell factors interact with the cytoplasmic region(s) of *Tir***? Mammalian cell factors that directly receive from *Tir*** the biochemical signal for actin filament formation will be identified. The proposed experiments may provide novel targets for therapeutic intervention during *EHEC*** infection, as well as provide insight into the general cellular mechanisms by which actin assembly is controlled.

7/3,AB/13 (Item 1 from file: 440)
 DIALOG(R) File 440:Current Contents Search(R)
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13059879 GENUINE ARTICLE#: 472JC NUMBER OF REFERENCES: 29
 TITLE: *Enteropathogenic*** *E.-coli**** *Tir*** *binds*** Nck to initiate actin pedestal formation in host cells
 AUTHOR(S): Gruenheid S; DeVinney R; Bladt F; Goosney D; Gelkop S; Gish GD; Pawson T; Finlay BB (REPRINT)
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 CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, 6174 Univ Blvd/Vancouver/BC V6T 1G3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1G3/Canada/; Mt Sinai Hosp, Samuel Lunenfeld Res Inst, /Toronto/ON M5G 1X5/Canada/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: NATURE CELL BIOLOGY, 2001, V3, N9 (SEP), P856-859
 PUBLISHER: MACMILLAN PUBLISHERS LTD, PORTERS SOUTH, 4 CRINAN ST, LONDON N1 9XW, ENGLAND

ISSN: 1465-7392

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia coli*** (*EPEC***) is a bacterial pathogen that causes infantile diarrhea worldwide(1). *EPEC*** injects a bacterial *protein***, *translocated*** *intimin*** *receptor*** (*Tir***), into the host-cell plasma membrane where it acts as a receptor for the bacterial outer membrane *protein***, *intimin*** (2). The interaction of *Tir*** and *intimin*** triggers a marked rearrangement of the host actin cytoskeleton into pedestals beneath adherent bacteria. On delivery into host cells, *EPEC*** *Tir*** is phosphorylated on tyrosine 474 of the intracellular carboxy-terminal domain, an event that is required for pedestal formation(3). Despite its essential role, the function of *Tir*** tyrosine phosphorylation has not yet been elucidated. Here we show that tyrosine 474 of *Tir*** directly *binds*** the host-cell adaptor *protein*** Nck, and that Nck is required for the recruitment of both neural Wiskott-Aldrich-syndrome *protein*** (N-WASP) and the actin-related *protein*** (Arp)2/3 complex to the *EPEC*** pedestal, directly linking *Tir*** to the cytoskeleton. Cells with null alleles of both mammalian Nck genes are resistant to the effects of *EPEC*** on the actin cytoskeleton. These results implicate Nck adaptors as host-cell determinants of *EPEC*** virulence.

7/3,AB/14 (Item 2 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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12995334 GENUINE ARTICLE#: 464PB NUMBER OF REFERENCES: 45

TITLE: *Intimin***-specific immune responses prevent bacterial colonization by the *attaching***-effacing*** pathogen Citrobacter rodentium

AUTHOR(S): Ghaem-Maghami M; Simmons CP (REPRINT); Daniell S; Pizza M; Lewis D; Frankel G; Dougan G

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CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Ctr Mol Microbiol & Infect, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Ctr Mol Microbiol & Infect, /London SW7 2AZ//England/; St George Hosp, Dept Infect Dis, /London SW17 0RE//England/; Chiron Vaccines Immunol Res Inst, /I-53100 Siena//Italy/

PUBLICATION TYPE: JOURNAL

PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N9 (SEP), P5597-5605

PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904
USA

ISSN: 0019-9567

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The formation of *attaching*** and *effacing*** (A/E) lesions on gut enterocytes is central to the pathogenesis of *enterohemorrhagic*** (*EHEC***) Escherichia coli***, *enteropathogenic*** E. coli*** (

*EPEC***), and the rodent pathogen *Citrobacter rodentium*. Genes encoding A/E lesion formation map to a chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Here we show that the LEE-encoded *proteins*** EspA, EspB, *Tir***, and *intimin*** are the targets of long-lived humoral immune responses in *C. rodentium*-infected mice. Mice infected with *C. rodentium* developed robust acquired immunity and were resistant to reinfection with wild-type *C. rodentium* or a *C. rodentium* derivative, DBS255(pCVD438), which expressed *intimin*** derived from *EPEC*** strain E2348/69. The receptor-binding domain of *intimin*** polypeptides*** is located within the carboxy-terminal 280 amino acids (Int280). Mucosal and systemic vaccination regimens using enterotoxin-based adjuvants were employed to elicit immune responses to recombinant Int280 alpha from *EPEC*** strain E2348/69. Mice vaccinated subcutaneously with Int280 alpha, in the absence of adjuvant, were significantly more resistant to oral challenge with DBS255(pCVD438) but not with wild-type *C. rodentium*. This type-specific immunity could not be overcome by employing an exposed, highly conserved domain of *intimin*** (Int(388-667)) as a vaccine. These results show that anti-*intimin*** immune responses can modulate the outcome (of a *C. rodentium* infection and support the use of *intimin*** as a component of a type-specific *EPEC*** or *EHEC*** vaccine.

7/3,AB/15 (Item 3 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)
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12970712 GENUINE ARTICLE#: 462BY NUMBER OF REFERENCES: 41
 TITLE: The *enterohaemorrhagic*** *Escherichia coli**** (serotype O157 : H7) *Tir*** molecule is not functionally interchangeable for its *enteropathogenic*** *E. coli**** (serotype O127 : H6) homologue
 AUTHOR(S): Kenny B (REPRINT)
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 CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: CELLULAR MICROBIOLOGY, 2001, V3, N8 (AUG), P499-510
 PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND
 ISSN: 1462-5814
 LANGUAGE: English DOCUMENT TYPE: ARTICLE
 ABSTRACT: A major virulence determinant of enteropathogenic *Escherichia coli* (*EPEC***) is the *Tir*** molecule that is translocated into the plasma membrane where it orchestrates cytoskeletal rearrangements. *Tir*** undergoes several phosphorylation events within host cells, with modification on a tyrosine essential for its actin-nucleating

function. The *EHEC*** (serotype O157:H7) *Tir*** homologue is not tyrosine phosphorylated implying that it uses an alternative mechanism to nucleate actin. This is supported in this study by the demonstration that *EHEC*** *Tir*** is unable to functionally substitute for its *EPEC*** homologue. Like *EPEC***, the *EHEC*** *Tir*** molecule is phosphorylated within host cells, with the actin-nucleating dysfunction correlated to an altered modification profile. In contrast to *EHEC*** *Tir***, the *EPEC*** *Tir*** molecule mediated actin nucleation whether delivered into host cells by either strain. Thus, it would appear that *EHEC*** encodes specific factor(s) that facilitate the correct modification of its *Tir*** molecule within host cells. Domain-swapping experiments revealed that the N-terminal, alpha-actinin *binding***, *Tir*** domains were functionally interchangeable, with both the actin-nucleating dysfunction and altered modification profiles linked to the *EHEC*** C-terminal *Tir*** domain. This tyrosine-independent modification process presumably confers an advantage to *EHEC*** O157:H7 and may contribute to the prevalence of this strain in *EHEC*** disease. The presented data are also consistent with *EPEC*** and *EHEC*** sharing non-phosphotyrosine phosphorylation event(s), with an important role for such modifications in *Tir*** function. An *EHEC***-induced phosphotyrosine dephosphorylation activity is also identified.

7/3,AB/16 (Item 4 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)
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12626083 GENUINE ARTICLE#: 422NQ NUMBER OF REFERENCES: 62
 TITLE: Site-directed mutagenesis of *intimin*** alpha modulates *intimin***
 -mediated tissue tropism and host specificity
 AUTHOR(S): Reece S; Simmons CP; Fitzhenry RJ; Matthews S; Phillips AD;
 Dougan G; Frankel G (REPRINT)
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 CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept
 Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll
 Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Royal Free
 Hosp, Ctr Paediat Gastroenterol, /London NW3 2QG//England/; Univ London
 Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7
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 PUBLICATION: MOLECULAR MICROBIOLOGY, 2001, V40, N1 (APR), P86-98
 PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE,
 OXON, ENGLAND
 ISSN: 0950-382X
 LANGUAGE: English DOCUMENT TYPE: ARTICLE
 ABSTRACT: The hallmark of *enteropathogenic*** (*EPEC***) and
 *enterohaemorrhagic*** (*EHEC***) Escherchia *coli*** adhesion to host

cells is intimate attachment leading to the formation of distinctive ' *attaching*** and *effacing***' lesions. This event is mediated, in part, by *binding*** of the bacterial adhesion molecule *intimin*** to a second bacterial *protein***, *Tir***, delivered by a type III secretion system into the host cell plasma membrane. The receptor- *binding*** activity of *intimin*** is localized to the C-terminal 280 amino acids (Int280) and at least five distinct *intimin*** types (alpha, beta, gamma, delta and epsilon) have been identified thus far. In addition to *binding*** to *Tir***, *intimin*** can also *bind*** to a component encoded by the host. The consequence of latter *intimin***- *binding*** activity may determine tissue tropism and host specificity. In this study we selected three amino acids in *intimin***, which are implicated in *Tir*** *binding***, for site-directed mutagenesis. We used the yeast two-hybrid system and gel overlays to study *intimin***- *Tir*** *protein*** interaction. In addition, the biological consequences of the mutagenesis was tested using a number of infection models (cultured epithelial cells, human intestinal explants and a mouse model). We report that while an 1237/897A substitution (positions numbered according to Int280 alpha /whole *intimin*** alpha) in *intimin*** or did not have any affect on its biological activity, a T255/914A substitution attenuated *intimin*** activity in vivo. In contrast, the mutation V252/911A affected tissue targeting in the human intestinal explant model and attenuated the biological activity of *intimin*** in the mouse model. This study provides the first clues of the molecular basis of how *intimin*** mediates tissue tropism and host specificity.

7/3,AB/17 (Item 5 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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12618914 GENUINE ARTICLE#: 423CT NUMBER OF REFERENCES: 44

TITLE: Recruitment of cytoskeletal and signaling *proteins*** to *enteropathogenic*** and *enterohemorrhagic*** Escherichia *coli*** pedestals

AUTHOR(S): Goosney DL; DeVinney R; Finlay BB (REPRINT)

AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237,Westbrook Bldg,6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Microbiol & Immunol, /Vancouver/BC V6T 1Z3/Canada/

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PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA

ISSN: 0019-9567

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) is a human pathogen that attaches to intestinal epithelial cells and causes chronic watery diarrhea. A close relative, *enterohemorrhagic*** E. *coli*** (*EHEC***), causes severe bloody diarrhea and hemolytic-uremic syndrome. Both pathogens insert a *protein***, *Tir***, into the host cell plasma membrane where it *binds*** *intimin***, the outer membrane ligand of *EPEC*** and *EHEC***. This interaction triggers a cascade of signaling events within the host cell and ultimately leads to the formation of an actin-rich pedestal upon which the pathogen resides. Pedestal formation is critical in mediating *EPEC***- and *EHEC***-induced diarrhea, get very little is known about its composition and organization. In *EPEC***, pedestal formation requires *Tir*** tyrosine 474 phosphorylation. In *EHEC*** *Tir*** is not tyrosine phosphorylated, yet the pedestals appear similar. The composition of the *EPEC*** and *EHEC*** pedestals was analyzed by examining numerous cytoskeletal, signaling, and adapter *proteins***. Of the 25 *proteins*** examined, only two, calpactin and CD44, were recruited to the site of bacterial attachment independently of *Tir***. Several others, including ezrin, talin, gelsolin, and tropomyosin, were recruited to the site of *EPEC*** attachment independently of *Tir*** tyrosine 474 phosphorylation but required *Tir*** in the host membrane. The remaining *proteins*** were recruited to the pedestal in a manner dependent on *Tir*** tyrosine phosphorylation or were not recruited at all. Differences were also found between the *EPEC*** and *EHEC*** pedestals: the adapter *proteins*** Grb2 and CrKII were recruited to the *EPEC*** pedestal but were absent in the *EHEC*** pedestal. These results demonstrate that although *EPEC*** and *EHEC*** recruit similar cytoskeletal *proteins***, there are also significant differences in pedestal composition.

7/3,AB/18 (Item 6 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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12205204 GENUINE ARTICLE#: 379JP NUMBER OF REFERENCES: 31

TITLE: Interaction of the *enteropathogenic*** Escherichia *coli*** *protein***, *translocated*** *intimin*** *receptor*** (*Tir***), with focal adhesion *proteins***

AUTHOR(S): Freeman NL; Zurawski DV; Chowrashi P; Ayooob JC; Huang LL; Mittal B; Sanger JM; Sanger JW (REPRINT)

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CORPORATE SOURCE: Univ Penn, Dept Cell & Dev Biol, /Philadelphia//PA/19104 (REPRINT); Univ Penn, Dept Cell & Dev Biol, /Philadelphia//PA/19104

PUBLICATION TYPE: JOURNAL

PUBLICATION: CELL MOTILITY AND THE CYTOSKELETON, 2000, V47, N4 (DEC), P 307-318

PUBLISHER: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK,
NY 10158-0012 USA

ISSN: 0886-1544

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: When *enteropathogenic*** Escherichia *coli*** (*EPEC***) attach and infect host cells, they induce a cytoskeletal rearrangement and the formation of cytoplasmic columns of actin filaments called pedestals. The attached *EPEC*** and pedestals move over the surface of the host cell in an actin-dependent reaction [Sanger et al., 1996: Cell Motil Cytoskeleton 34:279-287]. The discovery that *EPEC*** inserts the *protein***, *translocated*** *intimin*** *receptor*** (*Tir***), into the membrane of host cells, where it *binds*** the *EPEC*** outer membrane *protein***, *intimin*** [Kenny et al., 1997: Cell 91:511-520], suggests *Tir*** serves two functions: tethering the bacteria to the host cell and providing a direct connection to the host's cytoskeleton. The sequence of *Tir*** predicts a *protein*** of 56.8 kD with three domains separated by two predicted trans-membrane spanning regions. A GST-fusion *protein*** of the N-terminal 233 amino acids of *Tir*** (Tir1) *binds*** to alpha-actinin, talin, and vinculin from cell extracts. GST-Tir1 also coprecipitates purified forms of alpha-actinin, talin, and vinculin while GST alone does not *bind*** these three focal adhesion *proteins***. Biotinylated probes of these three *proteins*** also bound Tir1 cleaved from GST. Similar associations of alpha-actinin, talin, and vinculin were also detected with the C-terminus of *Tir***, i.e., Tir3, the last 217 amino acids. Antibody staining of *EPEC***-infected cultured cells reveals the presence of focal adhesion *proteins*** beneath the attached bacteria. Our experiments support a model in which the cytoplasmic domains of *Tir*** recruit, a number of focal adhesion *proteins*** that can *bind*** actin filaments to form pedestals. Since pedestals also contain villin, tropomyosin and myosin IT [Sanger et al., 1996: Cell Motil. Cytoskeleton 34:279-287], the pedestals appear to be a novel structure sharing properties of both focal adhesions and microvilli. Cell Motil. Cytoskeleton 47:307-318, 2000. (C) 2000 Wiley-Liss, Inc.

7/3,AB/19 (Item 7 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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11939983 GENUINE ARTICLE#: 348HD NUMBER OF REFERENCES: 14

TITLE: *Enteropathogenic*** E-*coli*** *translocated*** *intimin***
*receptor***, *Tir***, interacts directly with alpha-actinin

AUTHOR(S): Goosney DL; DeVinney R; Pfuetzner RA; Frey EA; Strynadka NC;
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CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T
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/Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Microbiol & Immunol, /Vancouver/BC V6T 1Z3/Canada/; Univ British Columbia, Dept Biochem & Mol Biol, /Vancouver/BC V6T 1Z3/Canada/

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LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) triggers a dramatic rearrangement of the host epithelial cell actin cytoskeleton to form an *attaching*** and *effacing*** lesion, or pedestal. The pathogen remains attached extracellularly to the host cell through the pedestal for the duration of the infection. At the tip of the pedestal is a bacterial *protein***, *Tir***, which is secreted from the bacterium into the host cell plasma membrane, where it functions as the receptor for an *EPEC*** outer membrane *protein***, *intimin*** [1]. Delivery of *Tir*** to the host cell results in its tyrosine phosphorylation, followed by *Tir***-intimin*** *binding***. *Tir*** is believed to anchor *EPEC*** firmly to the host cell, although its direct linkage to the cytoskeleton is unknown. Here, we show that *Tir*** directly *binds*** the cytoskeletal *protein*** alpha-actinin. alpha-actinin is recruited to the pedestal in a *Tir***-dependent manner and colocalizes with *Tir*** in infected host cells. *Binding*** is mediated through the amino terminus of *Tir***. Recruitment of alpha-actinin occurs independently of *Tir*** tyrosine phosphorylation. Recruitment of actin, VASP, and N-WASP, however, is abolished in the absence of this tyrosine phosphorylation. These results suggest that *Tir*** plays at least three roles in the host cell during infection: *binding*** *intimin*** on *EPEC***; mediating a stable anchor with alpha-actinin through its amino terminus in a phosphotyrosine-independent manner; and recruiting additional cytoskeletal *proteins*** at the carboxyl terminus in a phosphotyrosine-dependent manner. These findings demonstrate the first known direct linkage between extracellular *EPEC***, through the transmembrane *protein*** *Tir***, to the host cell actin cytoskeleton via alpha-actinin. (C) 2000 Elsevier Science Ltd. All rights reserved.

7/3,AB/20 (Item 8 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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11919468 GENUINE ARTICLE#: 346KM NUMBER OF REFERENCES: 26

TITLE: Human response to Escherichia coli O157 : H7 infection: Antibodies to secreted virulence factors

AUTHOR(S): Li YL; Frey E; Mackenzie AMR; Finlay BB (REPRINT)

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ISSN: 0019-9567

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ABSTRACT: Vaccination has been proposed for the prevention of disease due to *enterohemorrhagic*** Escherichia *coli*** (*EHEC***), but the immune response following human infection, including the choice of potential antigens, has not been well characterized. To study this, sera were obtained from five pediatric patients with acute diarrhea caused by E. coli O157:117 0, 8, and 60 days after hospitalization. These sera were used to examine the immune response to four different *EHEC*** virulence factors: *Tir*** (*translocated*** *intimin*** *receptor***, which is inserted into the host cell membrane), *intimin*** (bacterial outer membrane *protein*** which *binds*** to *Tir***), EspA (secreted *protein*** which forms filamentous structures on *EHEC*** surface), and EspB (inserted into the host membrane and cytoplasm). The response to O157:H7 lipopolysaccharide was also examined. Sera were assayed against purified recombinant *proteins*** using immunoblot analysis and by enzyme-linked immunosorbent assay to determine the sera's titers to each of the antigens in all patients. We found that there was little reaction to EspA EspB, and *intimin*** in the acute-phase sera, although there was some reactivity to *Tir***. By day 8, titers of antibody to all four virulence factors were present in all patients, with a very strong response against *Tir*** (up to a titer of 1:256,000), especially in hemolytic-uremic syndrome patients, and lesser strong responses to the other three antigens. The titer to the antigens 60 days after hospitalization was decreased but was still highest for *Tir***. These results suggest that there is a strong immune response to *Tir***, and to a lesser extent to the other three virulence factors, following *EHEC*** disease, indicating that these bacterial molecules are potential vaccine candidates for preventing *EHEC*** disease. They also suggest that bacterial virulence factors that are inserted into host cells during infection by type III secretion systems (*Tir*** or EspB) are still recognized by the host immune response.

7/3,AB/21 (Item 9 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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11896203 GENUINE ARTICLE#: 344RF NUMBER OF REFERENCES: 54

TITLE: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) attachment to

Searcher : Shears 308-4994

epithelial cells: exploiting the host cell cytoskeleton from the outside

AUTHOR(S): Celli J; Deng WY; Finlay BB (REPRINT)

AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237, Westbrook Bldg, 6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/

PUBLICATION TYPE: JOURNAL

PUBLICATION: CELLULAR MICROBIOLOGY, 2000, V2, N1 (FEB), P1-9

PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND

ISSN: 1462-5814

LANGUAGE: English DOCUMENT TYPE: REVIEW

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***), a leading cause of human infantile diarrhoea, is the prototype for a family of intestinal bacterial pathogens that induce *attaching*** and *effacing*** (A/E) lesions on host cells. A/E lesions are characterized by localized effacement of the brush border of enterocytes, intimate bacterial attachment and pedestal formation beneath the adherent bacteria. As a result of some recent breakthrough discoveries, *EPEC*** has now emerged as a fascinating paradigm for the study of host-pathogen interactions and cytoskeletal rearrangements that occur at the host cell membrane. *EPEC*** uses a type III secretion machinery to attach to epithelial cells, translocating its own receptor for intimate attachment, *Tir***, into the host cell, which then *binds*** to *intimin*** on the bacterial surface. Studies of *EPEC***-induced cytoskeletal rearrangements have begun to provide clues as to the mechanisms used by this pathogen to subvert the host cell cytoskeleton and signalling pathways. These findings have unravelled new ways by which pathogenic bacteria exploit host processes from the cell surface and have shed new light on how *EPEC*** might cause diarrhoea.

7/3, AB/22 (Item 10 from file: 440)

DIALOG(R) File 440: Current Contents Search(R)

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11889829 GENUINE ARTICLE#: 341UL NUMBER OF REFERENCES: 60

TITLE: Exploitation of host cells by *enteropathogenic*** Escherichia *coli***

AUTHOR(S): Vallance BA; Finlay BB (REPRINT)

AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, Room 237, Westbrook Bldg, 6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/

PUBLICATION TYPE: JOURNAL

PUBLICATION: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 2000, V97, N16 (AUG 1), P8799-8806

PUBLISHER: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC
20418 USA

ISSN: 0027-8424

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Microbial pathogens have evolved many ingenious ways to infect their hosts and cause disease, including the subversion and exploitation of target host cells. One such subversive microbe is enteropathogenic Escherichia coli (*EPEC***), A major cause of infantile diarrhea in developing countries, *EPEC*** poses a significant health threat to children worldwide, Central to *EPEC***-mediated disease is its colonization of the intestinal epithelium. After initial adherence, *EPEC*** causes the localized effacement of microvilli and intimately attaches to the host cell surface, forming characteristic *attaching*** and *effacing*** (A/E) lesions. Considered the prototype for a family of A/E lesion-causing bacteria, recent in vitro studies of *EPEC*** have revolutionized our understanding of how these pathogens infect their hosts and cause disease, Intimate attachment requires the type III-mediated secretion of bacterial *proteins***, several of which are translocated directly into the infected cell, including the bacteria's own receptor (*Tir***). *Binding*** to this membrane-bound, pathogen-derived *protein*** permits *EPEC*** to intimately attach to mammalian cells, The translocated *EPEC*** *proteins*** also activate signaling pathways within the underlying cell, causing the reorganization of the host actin cytoskeleton and the formation of pedestal-like structures beneath the adherent bacteria; This review explores what is known about *EPEC***'s subversion of mammalian cell functions and how this knowledge has provided novel insights into bacterial pathogenesis and microbe-host interactions, Future studies of A/E pathogens in animal models should provide further insights into how *EPEC*** exploits not only epithelial cells but other host cells, including those of the immune system, to cause diarrheal disease.

7/3,AB/23 (Item 11 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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11709866 GENUINE ARTICLE#: 322ZH NUMBER OF REFERENCES: 59

TITLE: Structural basis for recognition of the *translocated*** *intimin***
*receptor*** (*Tir***) by *intimin*** from *enteropathogenic***
Escherichia *coli***

AUTHOR(S): Batchelor M; Prasannan S; Daniell S; Reece S; Connerton I;
Bloomberg G; Dougan G; Frankel G (REPRINT); Matthews S

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CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept
Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll
Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ London

09/189415

Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7
2AZ//England/; Univ Nottingham, Div Food Sci, /Loughborough LE12
5RD/Leics/England/; Univ Bristol, Dept Biochem, /Bristol BS8
1TD/Avon/England/

PUBLICATION TYPE: JOURNAL

PUBLICATION: EMBO JOURNAL, 2000, V19, N11 (JUN 1), P2452-2464

PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND

ISSN: 0261-4189

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Intimin*** is a bacterial adhesion molecule involved in intimate attachment of *enteropathogenic*** and *enterohaemorrhagic*** Escherichia *coli*** to mammalian host cells. *Intimin*** targets the *translocated*** *intimin*** *receptor*** (*Tir***), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study we localized the *Tir***-*binding*** region of *intimin*** to the C-terminal 190 amino acids (Int190). We have also determined the region's high-resolution solution structure, which comprises an immunoglobulin domain that is intimately coupled to a novel C-type lectin domain. This fragment, which is necessary and sufficient for *Tir*** interaction, defines a new super domain in *intimin*** that exhibits striking structural similarity to the integrin-*binding*** domain of the Yersinia invasin and C-type lectin families. The extracellular portion of *intimin*** comprises an articulated rod of immunoglobulin domains extending from the bacterium surface, conveying a highly accessible 'adhesive tip' to the target cell. The interpretation of NMR-titration and mutagenesis data has enabled us to identify, for the first time, the *binding*** site for *Tir***, which is located at the extremity of the Int190 moiety.

7/3,AB/24 (Item 12 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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11236777 GENUINE ARTICLE#: 271PR NUMBER OF REFERENCES: 21

TITLE: Human colostrum and serum contain antibodies reactive to the *intimin***-*binding*** region of the *enteropathogenic*** Escherichia *coli*** *translocated*** *intimin*** *receptor***

AUTHOR(S): Sanches MI; Keller R; Hartland EL; Figueiredo DMM; Batchelor M; Martinez MB; Dougan G; Careiro-Sampaio MMS; Frankel G (REPRINT); Trabulsi LR

CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ Sao Paulo, Dept Immunol, /Sao Paulo//Brazil/; Univ Sao Paulo, Dept Anal Clin & Toxicol, /Sao Paulo//Brazil/

PUBLICATION TYPE: JOURNAL

PUBLICATION: JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, 2000, V30

Searcher : Shears 308-4994

, N1 (JAN), P73-77

PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA
19106-3621 USA

ISSN: 0277-2116

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Background: In Brazil, *enteropathogenic*** Escherichia *coli*** (*EPEC***) diarrhoea is endemic in young infants. A characteristic feature of *EPEC*** adhesion to host cells is intimate attachment leading to the formation of distinctive "*attaching*** and *effacing***" (A/E) lesions on mammalian cells. Two genes directly involved in intimate adhesion, *ene* and *tir***, encode the adhesion molecule *intimin*** and its translocated receptor *Tir***, respectively. The *intimin***-*binding*** domain of *Tir*** was recently mapped to the middle part of the *polypeptide*** (*Tir***-M), and the amino (*Tir***-N) and carboxy (*Tir***-C) termini were found to be located within infected host cells. Recently, it was shown that colostrum samples from mothers living in Sao Paulo contain IgA-class antibodies reactive with a number of *proteins*** associated with *EPEC*** virulence. It has also been shown that patients infected with verocytotoxin-producing E. coli O157 can produce antibodies to *Tir***. In the current study antibody responses to the different *Tir*** domains were analyzed in sera and colostrum samples collected in an *EPEC***-endemic area of Brazil.

Methods: Recombinant *Tir***, *Tir***-N, *Tir***-M, and *Tir***-C were expressed as His-tagged *protein*** in E. coli BL21a and purified on nickel columns. Western blot analysis was used to investigate colostrum IgA- and serum IgG-class antibodies reactive with the *Tir*** fragments.

Results: Anti-*Tir*** IgG antibodies were detected in the serum of children, with (63%) or without (50%) diarrhoea, Anti-*Tir*** IgA-class antibodies were detected in all the colostrum pools tested. With the use of both serum IgG- and colostrum IgA-class antibodies, an immunodominant domain of the *Tir***-*polypeptide***, *Tir*** M, was identified.

Conclusion: The *intimin***-*binding*** region of *Tir*** (*Tir***-M) is the immunodominant region of the *polypeptide*** in humans. Both serum IgG-class and colostrum IgA-class antibodies reacted predominantly with the *Tir***-M domain. (C) 2000 Lippincott Williams & Wilkins, Inc.

7/3,AB/25 (Item 13 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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11235993 GENUINE ARTICLE#: 271RC NUMBER OF REFERENCES: 16

09/189415

TITLE: Antibody response of patients infected with verocytotoxin-producing
Escherichia coli to *protein*** antigens encoded on the LEE locus
AUTHOR(S): Jenkins C; Chart H (REPRINT); Smith HR; Hartland EL; Batchelor M
; Delahay RM; Dougan G; Frankel G
AUTHOR(S) E-MAIL: hchart@phls.co.uk
CORPORATE SOURCE: Cent Publ Hlth Lab, Lab Enter Pathogens, 61 Colindale
Ave/London NW9 5HT//England/ (REPRINT); Cent Publ Hlth Lab, Lab Enter
Pathogens, /London NW9 5HT//England/; Univ London Sch Pharm, Dept
Biochem, /London SW7 2AY//England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF MEDICAL MICROBIOLOGY, 2000, V49, N1 (JAN), P97-101
PUBLISHER: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA
19106-3621 USA
ISSN: 0022-2615
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Sera from patients infected with verocytotoxin-producing
Escherichia coli (VTEC) O157, from patients with antibodies to E. coli
O157 lipopolysaccharide (LPS) and from healthy controls were examined
for antibodies to *proteins*** involved in expressing the *attaching***
and *effacing*** phenotype. After SDS-PAGE, purified recombinant
*intimin***, EspA-filament structural *protein***, translocated
*protein*** EspB and three separate domains of the *translocated***
*intimin*** *receptor*** (*Tir***) were tested for reaction with
patients' sera by immunoblotting. An ELISA was also used to detect
antibodies to *intimin*** in sera from E. coli O157 LPS
antibody-positive individuals. Seven of nine culture-positive patients
and one control patient had antibodies to EspA. Five of these patients
and two controls had serum antibodies to the *intimin***-*binding***
region of Tir, whereas none of the sera contained antibodies
*binding*** to either of the intracellular domains of *Tir***. By
immunoblotting, 10 of 14 culture-positive patients had antibodies to
the conserved region of *intimin***, eight of whom were infected with
E. coli O157 phage type 2. Thirty-six of 60 sera from culture-negative
but E. coli O157 LPS antibody-positive patients had antibodies to
*intimin*** as determined by ELISA. The secreted *proteins*** are
expressed in vivo during infection and are considered as pathogenic
markers. Antibodies to these *proteins*** may form the basis of a
serodiagnostic test for the detection of patients infected with VTEC
which carry the locus for the enterocyte effacement pathogenicity
island and provide an adjunct test to the established serological tests
based on VTEC LPS.

7/3,AB/26 (Item 14 from file: 440)
DIALOG(R) File 440:Current Contents Search(R)
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11216985 GENUINE ARTICLE#: 268UL NUMBER OF REFERENCES: 27

Searcher : Shears 308-4994

09/189415

TITLE: Hierarchy in the expression of the locus of enterocyte effacement genes of *enteropathogenic*** Escherichia *coli***
AUTHOR(S): Friedberg D; Umanski T; Fang YA; Rosenshine I (REPRINT)
AUTHOR(S) E-MAIL: ilanro@cc.huji.ac.il
CORPORATE SOURCE: Hebrew Univ Jerusalem, Dept Mol Genet, POB 12272/IL-91120 Jerusalem//Israel/ (REPRINT); Hebrew Univ Jerusalem, Dept Mol Genet, /IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Biotechnol, /IL-91120 Jerusalem//Israel/
PUBLICATION TYPE: JOURNAL
PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V34, N5 (DEC), P941-952
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND
ISSN: 0950-382X
LANGUAGE: English DOCUMENT TYPE: ARTICLE
ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) elicit changes in host cell morphology and cause actin rearrangement, a phenotype that has commonly been referred to as *attaching***/ *effacing*** (AE) lesions. The ability of *EPEC*** to induce AE lesions is dependent upon a type III *protein*** secretion/translocation system that is encoded by genes clustered in a 35.6 kb DNA segment, named the locus of enterocyte effacement (LEE). We used transcriptional fusions between the green fluorescent *protein*** (gfp) reporter gene and LEE genes rorf2, orf3, orf5, escJ, escV and eae, together with immunoblot analysis with antibodies against *Tir***, *intimin***, EspB and EspF, to analyse the genetic regulation of the LEE. The expression of all these LEE genes was strictly dependent upon the presence of a functional integration host factor (IHF). IHF *binds*** specifically upstream from the ler (orf1) promoter and appears to activate expression of ler, orf3, orf5 and rorf2 directly. The ler-encoded Ler *protein*** was involved in activating the expression of escJ, escV, *tir***, eae, espB and espF. Expression of both IHF and Ler was needed to elicit actin rearrangement associated with AE lesions. In conclusion, IHF directly activates the expression of the ler and rorf2 transcriptional units, and Ler in turn mediates the expression of the other LEE genes.

7/3,AB/27 (Item 15 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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11013552 GENUINE ARTICLE#: 245EX NUMBER OF REFERENCES: 60
TITLE: The *Tir***-*binding*** region of *enterohaemorrhagic*** Escherichia *coli*** *intimin*** is sufficient to trigger actin condensation after bacterial-induced host cell signalling
AUTHOR(S): Liu H; Magoun L; Luperchio S; Schauer DB; Leong JM (REPRINT)
AUTHOR(S) E-MAIL: john.leong@umassmed.edu
CORPORATE SOURCE: Univ Massachusetts, Dept Mol Genet & Microbiol, 55 Lake

Searcher : Shears 308-4994

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Ave N/Worcester//MA/01655 (REPRINT); Univ Massachusetts, Dept Mol Genet & Microbiol, /Worcester//MA/01655; MIT, Dept Bioengn & Environm Hlth, /Cambridge//MA/02139; MIT, Div Comparat Med, /Cambridge//MA/02139

PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V34, N1 (OCT), P67-81

PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND

ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enterohaemorrhagic*** Escherichia *coli*** (*EHEC***) has emerged as an important agent of diarrhoeal disease. Attachment to host cells, an essential step during intestinal colonization by *EHEC***, is associated with the formation of a highly organized cytoskeletal structure containing filamentous actin, termed an *attaching*** and *effacing*** (A/E) lesion, directly beneath bound bacteria. The outer membrane *protein*** *intimin*** is required for the formation of this structure, as is *Tir***, a bacterial *protein*** that is translocated into the host cell and is thought to function as a receptor for *intimin***. To understand *intimin*** function better, we fused *EHEC*** *intimin*** to a homologous *protein***, Yersinia pseudotuberculosis invasin, or to maltose-*binding*** *protein***. The N-terminal 539 amino acids of *intimin*** were sufficient to promote outer membrane localization of the C-terminus of invasin and, conversely, the N-terminal 489 amino acids of invasin were sufficient to promote the localization of the C-terminus of *intimin***. The C-terminal 181 residues of *intimin*** were sufficient to *bind*** mammalian cells that had been preinfected with an *enteropathogenic*** E. *coli*** strain that expresses *Tir*** but not *intimin***. *Binding*** of *intimin*** derivatives to preinfected cells correlated with *binding*** to recombinant *Tir*** *protein***. Finally, the 181-residue minimal *Tir***-*binding*** region of *intimin***, when purified and immobilized on latex beads, was sufficient to trigger A/E lesions on preinfected mammalian cells.

7/3,AB/28 (Item 16 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10970744 GENUINE ARTICLE#: 240GP NUMBER OF REFERENCES: 47

TITLE: Identification of Cest, a chaperone for the type III secretion of *Tir*** in *enteropathogenic*** Escherichia *coli***

AUTHOR(S): Elliott SJ; Hutcheson SW; Dubois MS; Mellies JL; Wainwright LA; Batchelor M; Frankel G; Knutton S; Kaper JB (REPRINT)

AUTHOR(S) E-MAIL: jkaper@umaryland.edu

CORPORATE SOURCE: Univ Maryland, Ctr Vaccine Dev, 685 W Baltimore St/Baltimore//MD/21201 (REPRINT); Univ Maryland, Ctr Vaccine Dev, /Baltimore//MD/21201; Univ Maryland, Dept Microbiol & Immunol,

Searcher : Shears 308-4994

09/189415

/Baltimore//MD/21201; Univ Maryland, Dept Mol Genet & Cell Biol,
/College Pk//MD/20742; Univ London Imperial Coll Sci Technol & Med,
Dept Biochem, /London SW7 2AZ//England/; Univ Birmingham, Inst Child
Hlth, /Birmingham B16 8ET/W Midlands/England/

PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N6 (SEP), P1176-1189

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OXON, ENGLAND

ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The locus of enterocyte effacement of *enteropathogenic**

Escherichia *coli*** encodes a type III secretion system, an outer
membrane *protein*** adhesin (*intimin***, the product of eae) and
*Tir***, a translocated *protein*** that becomes a host cell receptor
for *intimin***. Many type III secreted *proteins*** require
chaperones, which function to stabilize *proteins***, prevent
inappropriate *protein***-*protein*** interactions and aid in
secretion. An open reading frame located between fir and eae,
previously named orfU, was predicted to encode a *protein*** with
partial similarity to the Yersinia SycH chaperone. We examined the
potential of the orfU gene product to serve as a chaperone for *Tir***.
The orfU gene encoded a 15 kDa cytoplasmic *protein*** that
specifically interacted with *Tir*** as demonstrated by the yeast
two-hybrid assay, column *binding*** and coimmunoprecipitation
experiments. An orfU mutant was defective in *attaching***-*effacing***
lesion formation and *Tir*** secretion, but was unaffected in
expression of other virulence factors. OrfU:appeared to stabilize
*Tir*** levels in the cytoplasm, but was not absolutely necessary for
secretion of *Tir***. Based upon the physical similarities, phenotypic
characteristics and the demonstrated interaction with *Tir***, orfU is
redesignated as cest for the chaperone for E. coli secretion of *Tir***

7/3,AB/29 (Item 17 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10970743 GENUINE ARTICLE#: 240GP NUMBER OF REFERENCES: 57

TITLE: *Enteropathogenic*** Escherichia *coli*** *translocated***

*intimin*** *receptor***, *Tir***, requires a specific chaperone for
stable secretion

AUTHOR(S): Abe A; de Grado M; Pfuetzner RA; Sanchez-SanMartin C; DeVinney R
; Puente JL; Strynadka NCJ; Finlay BB (REPRINT)

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Bldg, 6174 Univ Blvd/Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ
British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ

Searcher : Shears 308-4994

09/189415

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1Z3/Canada/; Kitasato Inst, Minato Ku, /Tokyo 108//Japan/; Univ Nacl
Autonoma Mexico, Dept Mol Microbiol, /Cuernavaca 62250/Morelos/Mexico/

PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N6 (SEP), P1162-1175

PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE,
OXON, ENGLAND

ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) secretes several Esps (E. coli-secreted *proteins***) that are required for full virulence. Insertion of the bacterial *protein*** *Tir*** into the host epithelial cell membrane is facilitated by a type III secretion apparatus, and at least EspA and EspB are required for *Tir*** translocation. An *EPEC*** outer membrane *protein***, *intimin***, interacts with *Tir*** on the host membrane to establish intimate attachment and formation of a pedestal-like structure. In this study, we identified a *Tir*** chaperone, CestT, whose gene is located between *tir*** and eae (which encodes *intimin***). A mutation in cest abolished *Tir*** secretion into culture supernatants and significantly decreased the amount of *Tir*** in the bacterial cytoplasm. In contrast, this mutation did not affect the secretion of the Esp *proteins***. The level of *tir*** mRNA was not affected by the cest mutation, indicating that CestT acts at the post-transcriptional level. The cest mutant could not induce host cytoskeletal rearrangements, and displayed the same phenotype as the tir mutant. Gel overlay and GST pulldown assays demonstrated that CestT specifically interacts with *Tir***, but not with other Esp *proteins***. Furthermore, by using a series of *Tir*** deletion derivatives, we determined that the CestT *binding*** domain is located within the first 100 amino-terminal residues of *Tir***, and that the pool of *Tir*** in the bacterial cytoplasm was greatly reduced when this domain was disrupted. Interestingly, this domain was not sufficient for *Tir*** secretion, and at least the first 200 residues of *Tir*** were required for efficient secretion. Gel filtration studies showed that *Tir***-CestT forms a large multimeric complex. Collectively, these results indicate that CestT is a *Tir*** chaperone that may act as an anti-degradation factor by specifically *binding*** to its amino-terminus, forming a multimeric stabilized complex.

7/3,AB/30 (Item 18 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10910288 GENUINE ARTICLE#: 234HF NUMBER OF REFERENCES: 46

TITLE: A novel chromosomal locus of *enteropathogenic*** Escherichia *coli*** (*EPEC***), which encodes a bfpT-regulated chaperone-like

Searcher : Shears 308-4994

*protein***, TrcA, involved in microcolony formation by *EPEC***
 AUTHOR(S): Tobe T (REPRINT); Tatsuno I; Katayama E; Wu CY; Schoolnik GK;
 Sasakawa C
 AUTHOR(S) E-MAIL: torutobe@ims.u-tokyo.ac.jp
 CORPORATE SOURCE: Univ Tokyo, Minato Ku, 4-6-1 Shirokanedai/Tokyo
 1080071//Japan/ (REPRINT); Univ Tokyo, Minato Ku, /Tokyo
 1080071//Japan/; Univ Tokyo, Minato Ku, /Tokyo 1080071//Japan/;
 Stanford Univ, Sch Med, /Stanford//CA/94305; Osaka Univ, Dept Bacterial
 Toxinol, /Suita/Osaka 565/Japan/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V33, N4 (AUG), P741-752
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 OXON, ENGLAND
 ISSN: 0950-382X
 LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The bfpTVW operon, also known as the per operon, of
 *enteropathogenic*** Escherichia *coli*** (*EPEC***) is required for
 the transcriptional activation of the bfp operon, which encodes the
 major subunit and assembly machinery of bundle-forming pill (BFP). An
 immobilized T7-tagged BfpT fusion *protein*** that *binds***
 specifically to upstream promoter sequences of bfpA and eae was used to
 'fish out' from a promoter library other *EPEC*** chromosomal fragments
 that are bound by the BfpT *protein***. After screening for promoters
 exhibiting bfpTVW-dependent expression, one was identified that was
 positively regulated by bfpTVW and that is not present in the
 chromosomes of two non-virulent E. coli laboratory strains, DH5 alpha
 and HB101. Further analysis of this positively regulated promoter in
 *EPEC*** showed that it resided within a 4.9 kb sequence that is not
 present in E. coli K12. This locus, located downstream of the potB
 gene, was found to contain four open reading frames (ORFs):
 bfpTVW-activated promoter was localized upstream of ORF1. An ORF1
 knockout mutant produced less of the BFP structural subunit (BfpA) and
 formed smaller than normal adherent microcolonies on cultured
 epithelial cells; however, this mutation did not affect bfp
 transcription. An ORF1-His6 fusion *protein*** specifically bound the
 preprocessed and mature forms of the BfpA *protein*** and thus appears
 to stabilize the former within the cytoplasmic compartment. ORF1
 therefore is a newly isolated *EPEC*** chromosomal gene that encodes a
 chaperone-like *protein*** involved in the production of BFP. Hence,
 ORF1 was designated trcA (bfpT-regulated chaperone-like *protein***
 gene). The TrcA *protein*** also specifically bound 39 kDa and *90***
 *kDa*** *proteins*** that are expressed by *EPEC*** but not by E. coli
 K12. The *90*** *kDa*** *protein*** was revealed to be *intimin***, a
 *protein*** product of the eae gene, which is required for the *EPEC***
 *attaching***/*effacing*** phenotype, suggesting a direct interaction
 of TrcA with *intimin*** in the cytoplasmic compartment.

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7/3,AB/31 (Item 19 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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10749914 GENUINE ARTICLE#: 219LV NUMBER OF REFERENCES: 24
TITLE: Role of bacterial *intimin*** in colonic hyperplasia and
inflammation
AUTHOR(S): Higgins LM (REPRINT); Frankel G; Connerton I; Goncalves NS;
Dogan G; MacDonald TT
CORPORATE SOURCE: St Bartholomews & Royal London Sch Med & Dent, Dept
Paediat Gastroenterol, /London EC1A 7BE//England/ (REPRINT); St
Bartholomews & Royal London Sch Med & Dent, Dept Paediat Gastroenterol,
/London EC1A 7BE//England/; Univ London Imperial Coll Sci Technol &
Med, Dept Biochem, /London SW7 2AZ//England/; Univ Nottingham, Div Food
Sci, /Loughborough LE12 5RD/Leics/England/
PUBLICATION TYPE: JOURNAL
PUBLICATION: SCIENCE, 1999, V285, N5427 (JUL 23), P588-591
PUBLISHER: AMER ASSOC ADVANCEMENT SCIENCE, 1200 NEW YORK AVE, NW,
WASHINGTON, DC 20005 USA
ISSN: 0036-8075

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) cells adhere
to gut epithelial cells through *intimin*** alpha: the ligand for a
bacterially derived epithelial transmembrane *protein*** called the
*translocated*** *intimin*** *receptor***, Citrobacter rodentium
colonizes the mouse colon in a similar fashion and uses a different
*intimin***: *intimin*** beta. *Intimin*** alpha was found to
costimulate submitogenic signals through the T cell receptor. Dead
*intimin*** beta(+) C. rodentium, *intimin*** a-transfected C.
rodentium or E. coli strain K12, and *EPEC*** induced mucosal
hyperplasia identical to that caused by C. rodentium live infection, as
well as a massive T helper cell-type 1 immune response in the colonic
mucosa, Mutation of cysteine-937 of *intimin*** to alanine reduced
costimulatory activity in vitro and prevented immunopathology in vivo.
The mucosal changes elicited by C. rodentium were
interferon-gamma-dependent. Immunopathology induced by *intimin***
enables the bacteria to promote conditions that are favorable for
increased microbial colonization.

7/3,AB/32 (Item 20 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
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10475977 GENUINE ARTICLE#: 187BW NUMBER OF REFERENCES: 26
TITLE: *Binding*** of *intimin*** from *enteropathogenic*** Escherichia
*coli*** to *Tir*** and to host cells
AUTHOR(S): Hartland EL; Batchelor M; Delahay RM; Hale C; Matthews S; Dogan

Searcher : Shears 308-4994

09/189415

G; Knutton S; Connerton I; Frankel G (REPRINT)

AUTHOR(S) E-MAIL: g.frankel@ic.ac.uk

CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AZ//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AZ//England/; Univ Birmingham, Inst Child Hlth, /Birmingham B4 6NH/W Midlands/England/; Inst Food Res, Reading Lab, /Reading RG6 6BZ/Berks/England/; Univ Nottingham, Div Food Sci, /Loughborough LE12 5RD/Leics/England/

PUBLICATION TYPE: JOURNAL

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V32, N1 (APR), P151-158

PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND

ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) induce characteristic *attaching*** and *effacing*** (A/E) lesions on epithelial cells. This event is mediated, in part, by *binding*** of the bacterial outer membrane *protein***, *intimin***, to a second *EPEC*** *protein***, *Tir*** (*translocated*** *intimin*** *receptor***), which is exported by the bacteria and integrated into the host cell plasma membrane. In this study, we have localized the *intimin***-*binding*** domain of *Tir*** to a central 107-amino-acid region, designated *Tir***-M. We provide evidence that both the amino- and carboxy-termini of *Tir*** are located within the host cell. In addition, using immunogold labelling electron microscopy, we have confirmed that *intimin*** can *bind*** independently to host cells even in the absence of *Tir***. This *Tir***-independent interaction and the ability of *EPEC*** to induce A/E lesions requires an intact lectinlike module residing at the carboxy-terminus of the *intimin*** *polypeptide***. Using the yeast two-hybrid system and gel overlays, we show that *intimin*** can *bind*** both *Tir*** and *Tir***-M even when the lectin-like domain is disrupted. These data provide strong evidence that *intimin*** interacts not only with *Tir*** but also in a lectinlike manner with a host cell *intimin*** receptor.

7/3,AB/33 (Item 21 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10430360 GENUINE ARTICLE#: 182MT NUMBER OF REFERENCES: 46

TITLE: Structure of the cell-adhesion fragment of *intimin*** from *enteropathogenic*** Escherichia *coli***

AUTHOR(S): Kelly G; Prasannan S; Daniell S; Fleming K; Frankel G; Dougan G; Connerton I; Matthews S (REPRINT)

AUTHOR(S) E-MAIL: s.j.matthews@ic.ac.uk

Searcher : Shears 308-4994

09/189415

CORPORATE SOURCE: Univ London Imperial Coll Sci Technol & Med, Dept Biochem, Exhibit Rd/London SW7 2AY//England/ (REPRINT); Univ London Imperial Coll Sci Technol & Med, Dept Biochem, /London SW7 2AY//England/; Univ London Imperial Coll Sci Technol & Med, Ctr Struct Biol, /London SW7 2AY//England/; AFRC, Reading Lab, /Reading RG6 6BZ/Berks/England/; Univ London Imperial Coll Sci Technol & Med, Wellcome Ctr Infect Dis, /London SW7 2AY//England/

PUBLICATION TYPE: JOURNAL

PUBLICATION: NATURE STRUCTURAL BIOLOGY, 1999, V6, N4 (APR), P313-318

PUBLISHER: NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA

ISSN: 1072-8368

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) induce gross cytoskeletal rearrangement within epithelial cells, immediately beneath the attached bacterium. The C-terminal 280 amino acid residues of *intimin*** (Int280; 30.1 kDa), a bacterial cell-adhesion molecule, mediate the intimate bacterial host-cell interaction. Recently, interest in this process has been stimulated by the discovery that the bacterial *intimin*** receptor *protein*** (*Tir***) is translocated into the host cell membrane, phosphorylated, and after *binding*** *intimin*** triggers the intimate attachment. Using multidimensional nuclear magnetic resonance (NMR) and combining perdeuteration with site-specific protonation of methyl groups, we have determined the global fold of Int280. This represents one of the largest, non-oligomeric *protein*** structures to be determined by NMR that has not been previously resolved by X-ray crystallography, Int280 comprises three domains; two immunoglobulin-like domains and a C-type lectinlike module, which define a new family of bacterial adhesion molecules. These findings also imply that carbohydrate recognition may be important in *intimin***-mediated cell adhesion.

7/3,AB/34 (Item 22 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10323436 GENUINE ARTICLE#: 170EB NUMBER OF REFERENCES: 33

TITLE: Phosphorylation of tyrosine 474 of the *enteropathogenic*** Escherichia *coli*** (*EPEC***) *Tir*** receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications

AUTHOR(S): Kenny B (REPRINT)

AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk

CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/

PUBLICATION TYPE: JOURNAL

Searcher : Shears 308-4994

09/189415

PUBLICATION: MOLECULAR MICROBIOLOGY, 1999, V31, N4 (FEB), P1229-1241
PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE,
OXON, ENGLAND

ISSN: 0950-382X

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The *enteropathogenic*** Escherichia *coli*** (*EPEC***) *Tir***
*protein*** becomes tyrosine phosphorylated in host cells and displays
an increase in apparent molecular mass. The interaction of *Tir*** with
the *EPEC*** outer membrane *protein***, *intimin***, triggers actin
nucleation beneath the adherent bacteria. The *enterohaemorrhagic*** E.
*coli*** 0157:H7 (*EHEC***) *Tir*** molecule is not tyrosine
phosphorylated. In this paper, *Tir*** tyrosine phosphorylation is
shown to be essential for actin nucleation activity, but not for the
increase in apparent molecular mass observed in target cells. Tyrosine
phosphorylation had no role in *Tir*** molecular mass shift, indicating
additional host modifications. Analysis of *Tir*** intermediates
indicates that tyrosine-independent modification functions to direct
*Tir***'s correct insertion from the cytoplasm into the host membrane.
Deletion analysis identified *Tir*** domains participating in
translocation, association with the host membrane, modification and
antibody recognition. *Intimin*** was found to *bind*** a 55-amino-acid
region (TIBA) within *Tir*** that topological and sequence analysis
suggests is located in an extracellular loop. Homologous TIBA sequences
exist in integrins, which also *bind*** *intimin***. Collectively, this
study provides definitive evidence for the importance of tyrosine
phosphorylation for *EPEC*** *Tir*** function and reveals differences
in the pathogenicity of *EPEC*** and *EHEC***, The data also suggest a
mechanism for *Tir*** insertion into the host membrane, as well as
providing clues to the mode of *intimin***-integrin interaction.

7/3,AB/35 (Item 23 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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08563608 GENUINE ARTICLE#: XF631 NUMBER OF REFERENCES: 30

TITLE: *Intimin***-dependent *binding*** of *enteropathogenic***

Escherichia *coli*** to host cells triggers novel signaling events,
including tyrosine phosphorylation of phospholipase C-gamma 1

AUTHOR(S): Kenny B; Finlay BB (REPRINT)

CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB, ROOM 237 WESBROOK
BLDG, 6174 UNIV BLVD/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV
BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/

PUBLICATION TYPE: JOURNAL

PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N7 (JUL), P2528-2536

PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW,
WASHINGTON, DC 20005-4171

ISSN: 0019-9567

Searcher : Shears 308-4994

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** Escherichia *coli*** (*EPEC***) interactions with HeLa epithelial cells induced the tyrosine phosphorylation of a host *protein*** of approximately 150 kDa, Hp150. Phosphorylation of this *protein*** hand was dependent on the interaction of the *EPEC*** *protein*** *intimin*** with epithelial cell surfaces and was correlated with pedestal formation, Hp150 phosphorylation was specifically inhibited by the addition of cytochalasin D, an inhibitor of actin polymerization, although this appeared to be an indirect effect preventing interaction of *intimin*** with its receptor, tyrosine-phosphorylated *Hp90***, and thus triggering Hp150 phosphorylation. This suggests the involvement of an actin-based movement of membrane-bound tyrosine-phosphorylated *Hp90*** to allow its interaction with *intimin***. Analysis of the tyrosine-phosphorylated Hp150 *protein*** demonstrated that it is heterogeneous in composition, with phospholipase C-gamma 1 (PLC-gamma 1) being a minor component. Activation of PLC-gamma 1 by tyrosine phosphorylation leads to inositol triphosphate and Ca²⁺ fluxes, events defected following *EPEC*** infection. *EPEC*** also induced tyrosine dephosphorylation of host *proteins***, including a 240-kDa host *protein*** (Hp240), following *EPEC*** infection, *Protein*** dephosphorylation appears to be a signaling event which occurs independently of *intimin***. Inhibition of host tyrosine dephosphorylation events by the addition of the tyrosine phosphatase inhibitor sodium vanadate did not prevent actin accumulation beneath the adherent bacteria. We conclude that *EPEC*** induces two sets of signaling events following infection, One set is dependent on *EPEC*** *proteins*** secreted by the type III secretion pathway (EspA and EspB) which induces *Hp90*** tyrosine phosphorylation and dephosphorylation of host phosphotyrosine *proteins***. The second set, which is also dependent on the first signaling events, requires *intimin*** interaction with its receptor, tyrosine-phosphorylated *Hp90***, to trigger Hp150 and PLC-gamma 1 tyrosine phosphorylation as well as pedestal formation. Inhibition of pedestal formation by tyrosine kinase inhibitors indicates an important role for tyrosine phosphorylation events during *EPEC*** subversion of host processes.

7/3,AB/36 (Item 24 from file: 440)

DIALOG(R) File 440:Current Contents Search(R)

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07453860 GENUINE ARTICLE#: UQ620 NUMBER OF REFERENCES: 33

TITLE: A PATHOGENIC BACTERIUM TRIGGERS EPITHELIAL SIGNALS TO FORM A FUNCTIONAL BACTERIAL RECEPTOR THAT MEDIATES ACTIN PSEUDOPOD FORMATION
AUTHOR(S): ROSENSHINE I; RUSCHKOWSKI S; STEIN M; REINSCHIED DJ; MILLS SD; FINLAY BB (Reprint)

CORPORATE SOURCE: UNIV BRITISH COLUMBIA, BIOTECHNOL LAB/VANCOUVER/BC V6T

09/189415

1Z3/CANADA/ (Reprint); UNIV BRITISH COLUMBIA,BIOTECHNOL
LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT BIOCHEM &
MOLEC BIOL/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA,DEPT
MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/; HEBREW UNIV
JERUSALEM,FAC MED,DEPT BIOTECHNOL &MOL GENET/IL-91120
JERUSALEM//ISRAEL/

PUBLICATION: EMBO JOURNAL, 1996, V15, N11 (JUN 3), P2613-2624

ISSN: 0261-4189

LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: *Enteropathogenic*** E. *coli*** (*EPEC***) belongs to a group of bacterial pathogens that induce actin accumulation beneath adherent bacteria. We found that *EPEC*** adherence to epithelial cells mediates the formation of fingerlike pseudopods (up to 10 μ m) beneath bacteria. These actin-rich structures also contain tyrosine phosphorylated host *proteins*** concentrated at the pseudopod tip beneath adherent *EPEC***. Intimate bacterial adherence (and pseudopod formation) occurred only after prior bacterial induction of tyrosine phosphorylation of an epithelial membrane *protein***, *Hp90***, which then associates directly with an *EPEC*** adhesin, *intimin***. These interactions lead to cytoskeletal nucleation and pseudopod formation. This is the first example of a bacterial pathogen that triggers signals in epithelial cells which activates receptor *binding*** activity to a specific bacterial ligand and subsequent cytoskeletal rearrangement.

7/3,AB/37 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotechnology Abs

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0239735 DBA Accession No.: 1999-09836 PATENT

Escherichia coli recombinant *intimin*** receptor *protein*** - useful for distinguishing between enteropathogenic and enterohemorrhagic infection and for therapy and diagnosis

AUTHOR: Finlay B B; Kenny B; Devinney R; Stein M

CORPORATE SOURCE: Vancouver, British Columbia, Canada.

PATENT ASSIGNEE: Univ.British-Columbia 1999

PATENT NUMBER: WO 9924576 PATENT DATE: 19990520 WPI ACCESSION NO.:

1999-337712 (1928)

PRIORITY APPLIC. NO.: US 65130 APPLIC. DATE: 19971112

NATIONAL APPLIC. NO.: WO 98CA1042 APPLIC. DATE: 19981110

LANGUAGE: English

ABSTRACT: A translocated Escherichia coli *intimin*** receptor *protein*** (I) that *binds*** *intimin*** is new. Also claimed are: a DNA sequence (II) encoding (I) and its complements, fragments and variants; DNA probes specific for (II); vectors encoding (II) and host cells containing them; (I)-specific polyclonal or monoclonal antibody; recombinant production of (I); a fusion *protein*** containing (I); a method for identifying modulators of (I); a method for differentiating

Searcher : Shears 308-4994

between *attaching*** and *effacing*** pathogens by contacting them with an anti-(I) antibody and an anti-phosphotyrosine antibody; drug delivery to (I)-containing cells using a cell delivery vehicle; kits for the detection of (I) and (II); and a method for inducing a cell-mediated immune response in cattle or humans to a *protein*** of interest by contacting a subject with an attenuated bacteria, where the bacterium lacks an EspA or EspB *protein***, and contains (II) in a fusion construct. The presence of (I) in a sample is indicative of enteropathogenic or enterohemorrhagic infection. (91pp)

Set	Items	Description
S8	735	AU=(FINLAY, B? OR FINLAY B?)
S9	268	AU=(KENNY, B? OR KENNY B?)
S10	33	AU=(DEVINNEY, R? OR DE VINNEY, R? OR DEVINNEY R? OR DE VIN- NEY R?)
S11	3355	AU=(STEIN, M? OR STEIN M?)
S12	4	S8 AND S9 AND S10 AND S11
S13	67	S8 AND (S9 OR S10 OR S11)
S14	18	S9 AND (S10 OR S11)
S15	7	S10 AND S11

- Author(s)

S20 52 (S8 OR S9 OR S10 OR S11 OR S13) AND S3
 S21 42 (S12 OR S14 OR S15 OR S20) NOT S6
 S22 22 RD (unique items)
 >>>No matching display code(s) found in file(s): 65, 113

22/3,AB/1 (Item 1 from file: 65)
 DIALOG(R)File 65:Inside Conferences
 (c) 2001 BLDSC all rts. reserv. All rts. reserv.

02302103 INSIDE CONFERENCE ITEM ID: CN024112431
 Molecular mechanisms of enteropathogenic E. coli: Signal transduction, pedestal formation, intimate contact, and diarrhea
 Finlay, B. B.; *Kenny, B."***; *Stein, M."***; Reinscheid, D.
 CONFERENCE: Enteropathogenic Escherichia coli-International symposium
 REVISTA DE MICROBIOLOGIA, 1996; VOL 27; SUPP 1 P: 95-98
 (np), 1996
 ISSN: 0001-3714
 LANGUAGE: English DOCUMENT TYPE: Conference Papers
 CONFERENCE EDITOR(S): Kaper, J. B.
 CONFERENCE LOCATION: Sao Paulo, Brazil
 CONFERENCE DATE: Aug 1995 (199508) (199508)

22/3,AB/2 (Item 2 from file: 65)
 DIALOG(R)File 65:Inside Conferences
 (c) 2001 BLDSC all rts. reserv. All rts. reserv.

01741681 INSIDE CONFERENCE ITEM ID: CN017738833

Enteropathogenic E. coli Exploitation of Host Epithelial Cells

Finlay, B. B.; Ruschkowski, S.; *Kenny, B."**"; *Stein, M."**

CONFERENCE: Microbial pathogenesis and immune response-Meeting; 2nd

ANNALS- NEW YORK ACADEMY OF SCIENCES, 1996; VOL 797 P: 26-31

New York Academy of Sciences, 1996

ISSN: 0077-8923 ISBN: 1573310166; 1573310174

LANGUAGE: English DOCUMENT TYPE: Conference Papers

CONFERENCE EDITOR(S): Ades, E. W.; Morse, S. A.; Rest, R. F.

CONFERENCE LOCATION: New York, NY

CONFERENCE DATE: Oct 1995 (199510) (199510)

22/3,AB/3 (Item 1 from file: 144)

DIALOG(R)File 144:Pascal

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14307127 PASCAL No.: 99-0513847

Type III secretion-dependent hemolytic activity of enteropathogenic Escherichia coli

WARAWA J; *FINLAY B B"***; *KENNY B"***

Department of Pathology and Microbiology, School of Medical Sciences, Bristol, United Kingdom; Biotechnology Laboratory, Vancouver, British Columbia, V6T 1Z3, Canada

Journal: Infection and immunity, 1999, 67 (10) 5538-5540

Language: English

Enteropathogenic Escherichia coli (EPEC) was found to exhibit a type III secretion-dependent, contact-mediated, hemolytic activity requiring the EspA, EspB, and EspD secreted *proteins"***. EspB and EspD display homology to pore-forming molecules. Our data suggest a mechanism to explain the requirement for all three Esp *proteins"*** in the transfer of EPEC *proteins"***, such as *Tir"***, into target cells.

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22/3,AB/4 (Item 2 from file: 144)

DIALOG(R)File 144:Pascal

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14080585 PASCAL No.: 99-0273444

Enteropathogenic Escherichia coli : cellular harassment

Host-microbe interactions: bacteria

*DEVINNEY R"***; KNOECHEL D G; *FINLAY B B"***

COSSART Pascale, ed; MILLER Jeff F, ed

Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada

Unite des Interactions Bacteries-Cellules, Institut Pasteur, 28 rue du Dr

Roux, 75015 Paris, France; University of California Los Angeles School of Medicine, Dept of Microbiology and Immunology; 10833 Le Conte Ave., Los Angeles, CA 90024, United States

Journal: Current opinion in microbiology, 1999, 2 (1) 83-88

Language: English

The mechanisms by which enteropathogenic *Escherichia coli* (EPEC) mediates diarrhea remain a mystery. Recently a number of interesting and at times surprising results have come from studying EPEC interactions with host cells. Identification and characterization of bacterial factors, including *Tir***, EspA, EspB and EspD, and host responses have expanded our grasp of the diverse effects of EPEC on host cells.

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22/3,AB/5 (Item 3 from file: 144)

DIALOG(R)File 144:Pascal

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13217033 PASCAL No.: 97-0484125

Characterization of two virulence proteins secreted by rabbit enteropathogenic *Escherichia coli*, EspA and EspB, whose maximal expression is sensitive to host body temperature

ABE A; *KENNY B***; *STEIN M***; FINLAY B B

Biotechnology, Laboratory, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada; Department of Bacteriology, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

Journal: Infection and immunity, 1997, 65 (9) 3547-3555

Language: English

Enteropathogenic *Escherichia coli* (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-I has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified EPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC 0127. The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-I EspB was identical to that of enterohemorrhagic *E. coli* serotype 026. Mutations in RDEC-I espA and espB revealed that the corresponding RDEC-I gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing EPEC espA or/and espB genes into RDEC-I mutant strains demonstrated that they were functionally interchangeable, although the EPEC proteins mediated higher levels of invasion. Furthermore, maximal

expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.

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22/3,AB/6 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal
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13078049 PASCAL No.: 97-0369601
Enteropathogenic Escherichia coli protein secretion is induced in response to conditions similar to those in the gastrointestinal tract
*KENNY B***; ABE A; *STEIN M***; FINLAY B B
Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, V6T-1Z3, Canada
Journal: Infection and immunity, 1997, 65 (7) 2606-2612
Language: English

The pathogenicity of enteropathogenic Escherichia coli (EPEC) is associated with the expression and secretion of specific bacterial factors. EspB is one such secreted protein which is required to trigger host signaling pathways resulting in effacement of microvilli and cytoskeletal rearrangements. These events presumably contribute to the ensuing diarrhea associated with EPEC infections. EPEC encounters several environmental changes and stimuli during its passage from the external environment into the host gastrointestinal tract. In this paper we show that the secretion of EspB is subject to environmental regulation, and maximal secretion occurs under conditions reminiscent of those in the gastrointestinal tract. Thus, secretion is maximal at 37 Degree C, pH 7, and physiological osmolarity. In addition, maximal secretion requires the presence of sodium bicarbonate and calcium and is stimulated by millimolar concentrations of Fe(NO SUB 3) SUB 3 . The secretion of the four other EPEC-secreted proteins appears to be modulated in a manner similar to that of EspB. Our results also show that secretion is not dependent on CO SUB z , as originally reported by Haigh et al. (FEMS Microbiol. Lett. 129: 63-67, 1995), but that CO SUB z more likely acts as a component of the medium buffering system, since CO SUB 2 dependence was abolished by the use of alternative buffers.

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22/3,AB/7 (Item 5 from file: 144)
DIALOG(R)File 144:Pascal
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10356299 PASCAL No.: 92-0559759

09/189415

Signal transduction between enteropathogenic Escherichia coli (EPEC) and epithelial cells : EPEC induces tyrosine phosphorylation of host cell *proteins*** to initiate cytoskeletal rearrangement and bacterial uptake

ROSENSHINE I; DONNENBERG M S; KAPER J B; *FINLAY B B***

Univ. British Columbia, Canadian Bacterial Diseases Network, biotechnology lab., Vancouver BC, Canada

Journal: EMBO journal, 1992, 11 (10) 3551-3560

Language: English

Upon attachment to cultured HeLa cells, enteropathogenic Escherichia coli (PEC) induces assembly of a complex cytoskeletal structure within the eucaryotic cell, localized beneath the afferent bacterium. In addition, EPEC induces its own internalization by non-phagocytic epithelial cells. We found that after binding to the epithelial cell surface, EPEC induces tyrosine phosphorylation of three eucaryotic *proteins***. The major phosphorylation substrate is a *90*** *kDa*** *protein*** (*Hp90***)

22/3,AB/8 (Item 1 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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12568712 GENUINE ARTICLE#: 416HT NUMBER OF REFERENCES: 56

TITLE: Enteropathogenic Escherichia coli mediates antiphagocytosis through the inhibition of PI 3-kinase-dependent pathways

AUTHOR(S): Celli J; Olivier M; *Finlay BB (REPRINT)***

AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z3/Canada/; Univ Laval, Infect Dis Unit, /Quebec City/PQ G1V 4G2/Canada/

PUBLICATION TYPE: JOURNAL

PUBLICATION: EMBO JOURNAL, 2001, V20, N6 (MAR 15), P1245-1258

PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND

ISSN: 0261-4189

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The extracellular pathogen enteropathogenic Escherichia coli (EPEC) uses a type III secretion system to inhibit its uptake by macrophages. We show that EPEC antiphagocytosis is independent of the *translocated*** *intimin*** *receptor*** *Tir*** and occurs by preventing F-actin polymerization required for bacterial uptake. EPEC-macrophage contact triggered activation of phosphatidylinositol (PI) 3-kinase, which was subsequently inhibited in a type III secretion-dependent manner. Inhibition of PI 3-kinase significantly reduced uptake of a secretion-deficient mutant, without affecting antiphagocytosis by the wild type, suggesting that EPEC blocks a PI 3-kinase-dependent phagocytic pathway. EPEC specifically inhibited Fc gamma receptor- but not CR3-receptor mediated phagocytosis of opsonized zymosan, We showed that EPEC inhibits PI 3-kinase activity rather than

its recruitment to the site of bacterial contact. Phagocytosis of a secretion mutant correlated with the association of PI 3-kinase with tyrosine-phosphorylated *proteins"*, which wild-type EPEC prevented. These results show that EPEC blocks its uptake by inhibiting a PI 3-kinase-mediated pathway, and translocates effectors other than *Tir"* to interfere with actin-driven host cell processes. This constitutes a novel mechanism of phagocytosis avoidance by an extracellular pathogen.

22/3,AB/9 (Item 2 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)
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12441711 GENUINE ARTICLE#: 404GZ NUMBER OF REFERENCES: 41
 TITLE: Enteropathogenic Escherichia coli (EPEC) *Tir"* receptor molecule does not undergo full modification when introduced into host cells by EPEC-independent mechanisms
 AUTHOR(S): *Kenny B (REPRINT)*"; Warawa J
 AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
 CORPORATE SOURCE: Sch Med Sci Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Sch Med Sci Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N3 (MAR), P1444-1453
 PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904
 USA
 ISSN: 0019-9567
 LANGUAGE: English DOCUMENT TYPE: ARTICLE
 ABSTRACT: Enteropathogenic Escherichia coli (EPEC), like many other gram-negative pathogens, encodes a type III secretion apparatus dedicated to the release of virulence-associated *proteins*". One such *protein"*, *Tir"*, is translocated into host cells, where it is modified by the addition of phosphate groups, resulting in a number of species with distinct molecular mass. One phosphorylation event, on tyrosine residue 474 of *Tir"*, does not contribute to shifts in molecular mass but is essential for its actin-nucleating function. The role of the nonphosphotyrosine related modifications is unknown. In this paper, we demonstrate, using three different approaches, that *Tir"* does not encode sufficient information to facilitate its complete modification when introduced into host cells in EPEC-independent mechanisms. Each system revealed that *Tir"* is a substrate for a host kinase whose action results in its partial modification to a form similar to one evident in EPEC-infected host cells. Further *Tir"* modification could not be induced by infecting cells with EPEC, suggesting that *Tir"* must be coexpressed with other EPEC factors to enable its full modification within host cells. One approach used Yersinia spp. to deliver *Tir"* into host cells, and

this system revealed that *Tir*** secretion and translocation can occur in the absence of the *Tir*** chaperone molecule, CseT (formerly known as OrfU). CseT was found to be an efficiency factor which was not required, unlike in EPEC, for *Tir*** stability, indicating that it may function to guide *Tir*** to the translocation apparatus or maintain it in a secretion-competent form.

22/3,AB/10 (Item 3 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)
 (c) 2001 Inst for Sci Info. All rts. reserv.

12287537 GENUINE ARTICLE#: 387GA NUMBER OF REFERENCES: 43
 TITLE: Targeting of an enteropathogenic Escherichia coli (EPEC) effector *protein*** to host mitochondria
 AUTHOR(S): *Kenny B (REPRINT)***; Jepson M
 AUTHOR(S) E-MAIL: B.Kenny@bristol.ac.uk
 CORPORATE SOURCE: Univ Bristol, Dept Pathol & Microbiol, Univ Walk/Bristol BS8 1TD/Avon/England/ (REPRINT); Univ Bristol, Dept Pathol & Microbiol, /Bristol BS8 1TD/Avon/England/; Univ Bristol, Cell Imaging Facil, /Bristol BS8 1TD/Avon/England/; Univ Bristol, Dept Biochem, /Bristol BS8 1TD/Avon/England/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: CELLULAR MICROBIOLOGY, 2000, V2, N6 (DEC), P579-590
 PUBLISHER: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 ONE, OXON, ENGLAND
 ISSN: 1462-5814
 LANGUAGE: English DOCUMENT TYPE: ARTICLE
 ABSTRACT: Many Gram-negative pathogens use a type III secretion apparatus to deliver effector molecules into host cells to subvert cellular processes in favour of the pathogen. Enteropathogenic Escherichia coli (EPEC) uses such a system to deliver the *Tir*** effector molecule into host cells. In this paper, we show that the gene upstream of *tir***, orf19, encodes an additional type III secreted effector *protein***. Orf19 is delivered into host cells by a mechanism independent of endocytosis, but dependent on EspB. Orf19 is targeted to host mitochondria, where it appears to interfere with the ability to maintain membrane potential. Although the precise role of Orf19 remains to be elucidated, its interaction with mitochondria suggests a possible role in the subversion of key functions of these organelles, such as energy production or control of cell death. This is the first example of a type III secreted *protein*** targeted to mitochondria; it is probable that homologues (present in EPEC and Shigella species) and other bacterial effectors will also target this organelle.

22/3,AB/11 (Item 4 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)

09/189415

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12271566 GENUINE ARTICLE#: 384LF NUMBER OF REFERENCES: 24

TITLE: *Tir*** tyrosine phosphorylation and pedestal formation are delayed
in enteropathogenic Escherichia coli sepZ :: TnpH mutant 30-5-1(3)

AUTHOR(S): *DeVinney R***; Nisan I; Ruschkowski S; Rosenshine I; *Finlay
BB (REPRINT)***

AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T
1Z3/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab,
/Vancouver/BC V6T 1Z3/Canada/; Hebrew Univ Jerusalem, Dept Mol Genet,
/IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Biotechnol,
/IL-91120 Jerusalem//Israel/; Hebrew Univ Jerusalem, Dept Clin
Microbiol, /IL-91120 Jerusalem//Israel/

PUBLICATION TYPE: JOURNAL

PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N1 (JAN), P559-563

PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904
USA

ISSN: 0019-9567

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Enteropathogenic Escherichia coli (EPEC) strain 30-5-1(3) has
been reported to form attaching and effacing (A/E) lesions without
*Tir*** tyrosine phosphorylation. In this study, we show that
30-5-1(3), which has a transposon insertion within the sepZ gene, forms
wild-type A/E lesions including *Tir*** tyrosine phosphorylation, but
at a slower rate. A/E lesion formation by 30-5-1(3) occurs without
detectable secretion of *Tir*** or other EPEC Esp secreted *proteins***

22/3,AB/12 (Item 5 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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12265208 GENUINE ARTICLE#: 385NH NUMBER OF REFERENCES: 84

TITLE: Gut feelings: enteropathogenic E-coli (EPEC) interactions with the
host

AUTHOR(S): Goosney DL (REPRINT); Gruenheid S; *Finlay BB***

AUTHOR(S) E-MAIL: bfinlay@interchange.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T
1W5/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab,
/Vancouver/BC V6T 1W5/Canada/; Univ British Columbia, Dept Microbiol &
Immunol, /Vancouver/BC V6T 1W5/Canada/

PUBLICATION TYPE: JOURNAL

PUBLICATION: ANNUAL REVIEW OF CELL AND DEVELOPMENTAL BIOLOGY, 2000, V16, P
173-+

PUBLISHER: ANNUAL REVIEWS, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA
94303-0139 USA

09/189415

ISSN: 1081-0706

LANGUAGE: English DOCUMENT TYPE: REVIEW

ABSTRACT: Enteropathogenic Escherichia coli (EPEC) is a gram-negative bacterial pathogen that adheres to human intestinal epithelial cells, resulting in watery, persistent diarrhea. It subverts the host cell cytoskeleton, causing a rearrangement of cytoskeletal components into a characteristic pedestal structure underneath adherent bacteria. In contrast to other intracellular pathogens that affect the actin cytoskeleton from inside the host cytoplasm, EPEC remains extracellular and transmits signals through the host cell plasma membrane via direct injection of virulence factors by a "molecular syringe," the bacterial type III secretion system. One injected factor is *Tir"*, which functions as the plasma membrane receptor for EPEC adherence. *Tir"* directly links extracellular EPEC through the epithelial membrane and firmly anchors it to the host cell actin cytoskeleton, thereby initiating pedestal formation. In addition to stimulating actin nucleation and polymerization in the host cell, EPEC activates several other signaling pathways that lead to tight junction disruption, inhibition of phagocytosis, altered ion secretion, and immune responses. This review summarizes recent developments in our understanding of EPEC pathogenesis and discusses similarities and differences between EPEC pedestals, focal contacts, and Listeria monocytogenes actin tails.

22/3,AB/13 (Item 6 from file: 440)

DIALOG(R)File 440:Current Contents Search(R)

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10665713 GENUINE ARTICLE#: 209MM NUMBER OF REFERENCES: 122

TITLE: Enteropathogenic Escherichia coli: a pathogen that inserts its own receptor into host cells

AUTHOR(S): *DeVinney R"*; Gauthier A; Abe A; *Finlay BB (REPRINT)"

AUTHOR(S) E-MAIL: bfinlay@unixg.ubc.ca

CORPORATE SOURCE: Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z4/Canada/ (REPRINT); Univ British Columbia, Biotechnol Lab, /Vancouver/BC V6T 1Z4/Canada/; Kitasato Inst, Minato Ku, /Tokyo 108//Japan/

PUBLICATION TYPE: JOURNAL

PUBLICATION: CELLULAR AND MOLECULAR LIFE SCIENCES, 1999, V55, N6-7 (JUN), P 961-976

PUBLISHER: BIRKHAUSER VERLAG AG, VIADUKSTRASSE 40-44, PO BOX 133, CH-4010 BASEL, SWITZERLAND

ISSN: 1420-682X

LANGUAGE: English DOCUMENT TYPE: REVIEW

ABSTRACT: Enteropathogenic Escherichia coli (EPEC) is a major cause of infant diarrhea, killing hundreds of thousands of children per year worldwide. Intimate attachment to the host cell leading to the

formation of actin-rich pedestals beneath the adhering bacteria is an essential feature of EPEC pathogenesis. EPEC attaches to host cells via the outer membrane adhesin, intimin. It was recently shown that EPEC inserts its own receptor for intimate adherence, *Tir*** (*translocated*** *intimin*** *receptor***) into the host cell membrane. The focus of this review is on the discovery and characterization of this novel receptor, and our current understanding of its role in pedestal formation. Gram-negative bacterial secretion systems, including type III secretion systems, are reviewed and discussed in the context of *Tir*** delivery into the host cell membrane. The relationship and relevance of in vitro models compared to the actual in vivo situation is essential to understanding disease. We have critically reviewed the use of animal models in studying EPEC infection. Elucidating the function of *Tir*** will contribute to our understanding of how EPEC mediates disease.

22/3,AB/14 (Item 7 from file: 440)
 DIALOG(R) File 440:Current Contents Search(R)
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08996050 GENUINE ARTICLE#: YG492 NUMBER OF REFERENCES: 31
 TITLE: Enteropathogenic E-coli (EPEC) transfers its receptor for intimate adherence into mammalian cells
 AUTHOR(S): *Kenny B***; *DeVinney R***; *Stein M***; Reinscheid DJ; Frey EA; *Finlay BB (REPRINT)***
 CORPORATE SOURCE: UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/(REPRINT); UNIV BRITISH COLUMBIA,DEPT BIOCHEM & MOL BIOL, DEPT MICROBIOL & IMMUNOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: CELL, 1997, V91, N4 (NOV 14), P511-520
 PUBLISHER: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138
 ISSN: 0092-8674
 LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Enteropathogenic E. coli (EPEC) belongs to a group of bacterial pathogens that induce epithelial cell actin rearrangements resulting in pedestal formation beneath adherent bacteria. This requires the secretion of specific virulence *proteins*** needed for signal transduction and intimate adherence. EPEC interaction induces tyrosine phosphorylation of a *protein*** in the host membrane, *Hp90***, which is the receptor for the EPEC outer membrane *protein***, intimin. *Hp90***-intimin interaction is essential for intimate attachment and pedestal formation. Here, we demonstrate that *Hp90*** is actually a bacterial *protein*** (*Tir***). Thus, this bacterial pathogen inserts its own receptor into mammalian cell surfaces, to which it then adheres to trigger additional host signaling events and actin nucleation. It is

also tyrosine-phosphorylated upon transfer into the host cell.

22/3,AB/15 (Item 8 from file: 440)
 DIALOG(R)File 440:Current Contents Search(R)
 (c) 2001 Inst for Sci Info. All rts. reserv.

08760754 GENUINE ARTICLE#: XT420 NUMBER OF REFERENCES: 40
 TITLE: Characterization of two virulence proteins secreted by rabbit enteropathogenic Escherichia coli, EspA and EspB, whose maximal expression is sensitive to host body temperature
 AUTHOR(S): Abe P; *Kenny B***; *Stein M***; Finlay BB (REPRINT)
 CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB, ROOM 237, WESBROOK BLDG, 6174 UNIV BLVD/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; KITASATO INST,DEPT BACTERIOL, MINATO KU/TOKYO 108//JAPAN/
 PUBLICATION TYPE: JOURNAL
 PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N9 (SEP), P3547-3555
 PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
 ISSN: 0019-9567
 LANGUAGE: English DOCUMENT TYPE: ARTICLE
 ABSTRACT: Enteropathogenic Escherichia coli (EPEC) and rabbit EPEC (RDEC-1) cause unique histopathological features on intestinal mucosa, including attaching/effacing (A/E) lesions. Due to the human specificity of EPEC, RDEC-1 has been used as an animal model to study EPEC pathogenesis. At least two of the previously identified FPEC-secreted proteins, EspA and EspB, are required for triggering host epithelial signal transduction pathways, intimate adherence, and A/E lesions. However, the functions of these secreted proteins and their roles in pathogenesis have not been characterized. To investigate the function of EspA and EspB in RDEC-1, the espA and espB genes were cloned and their sequences were compared to that of EPEC O127. The EspA proteins showed high similarity (88.5% identity), while EspB was heterogeneous in internal regions (69.8% identity). However, RDEC-1 EspB was identical to that of enterohemorrhagic E. coli serotype O26. Mutations in RDEC-1 espA and espB revealed that the corresponding RDEC-1 gene products are essential for triggering of host signal transduction pathways and invasion into HeLa cells. Complementation with plasmids containing FPEC espA or/and espB genes into RDEC-1 mutant strains demonstrated that they were functionally interchangeable, although the FPEC proteins mediated higher levels of invasion. Furthermore, maximal expression of RDEC-1 and EPEC-secreted proteins occurred at their respective host body temperatures, which may contribute to the lack of EPEC infectivity in rabbits.

22/3,AB/16 (Item 9 from file: 440)

09/189415

DIALOG(R)File 440:Current Contents Search(R)

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07901289 GENUINE ARTICLE#: VR931 NUMBER OF REFERENCES: 56

TITLE: Characterization of EspC, a 110-kilodalton protein secreted by enteropathogenic *Escherichia coli* which is homologous to members of the immunoglobulinA protease-like family of secreted proteins

AUTHOR(S): *Stein M***; *Kenny B***; *Stein MA***; Finlay BB

CORPORATE SOURCE: UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/ (REPRINT); UNIV BRITISH COLUMBIA, DEPT BIOCHEM & MOL BIOL, BIOTECHNOL LAB/VANCOUVER/BC V6T 1Z3/CANADA/; UNIV BRITISH COLUMBIA, DEPT MICROBIOL & IMMUNOL/VANCOUVER/BC V6T 1Z3/CANADA/

PUBLICATION TYPE: JOURNAL

PUBLICATION: JOURNAL OF BACTERIOLOGY, 1996, V178, N22 (NOV), P6546-6554

PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171

ISSN: 0021-9193

LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Enteropathogenic *Escherichia coli* (EPEC) secretes at least five proteins. Two of these proteins, EspA and EspB (previously called EaeB), activate signal transduction pathways in host epithelial cells. While the role of the other three proteins (39, 40, and 110 kDa) remains undetermined, secretion of all five proteins is under the control of pcrA, a known positive regulator of several EPEC virulence factors. On the basis of amino-terminal protein sequence data, we cloned and sequenced the gene which encodes the 110-kDa secreted protein and examined its possible role in EPEC signaling and interaction with epithelial cells. In accordance with the terminology used for cspA, and espB, we called this gene espC, for EPEC-secreted protein C. We found significant homology between the predicted EspC protein sequence and a family of immunoglobulin A (IgA) protease-like proteins which are widespread among pathogenic bacteria. Members of this protein family are found in avian pathogenic *Escherichia coli* (Tsh), *Haemophilus influenzae* (Hap), and *Shigella flexneri* (SepA). Although these proteins and EspC do not encode IgA protease activity, they have considerable homology with IgA protease from *Neisseria gonorrhoeae* and *H. influenzae* and appear to use a export system for secretion. We found that genes homologous to espC also exist in other pathogenic bacteria which cause attaching and effacing lesions, including *Hafnia alvei* biotype 19982, *Citrobacter freundii* biotype 4280, and rabbit diarrheagenic *E. coli* (RDEC-1). Although these strains secrete various proteins similar in molecular size to the proteins secreted by EPEC, we did not detect secretion of a 110-kDa protein by these strains. To examine the possible role of EspC in EPEC interactions with epithelial cells, we constructed a deletion mutant in espC by allelic exchange and characterized the mutant by standard tissue culture assays. We found that EspC is not necessary for mediating EPEC-induced signal transduction in HeLa epithelial cells and does not play a role in

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adherence or invasion of tissue culture cells.

22/3,AB/17 (Item 10 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2001 Inst for Sci Info. All rts. reserv.

05272639 GENUINE ARTICLE#: MY484 NUMBER OF REFERENCES: 28
TITLE: A DIARRHEAL PATHOGEN, ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC),
TRIGGERS A FLUX OF INOSITOL PHOSPHATES IN INFECTED EPITHELIAL CELLS
AUTHOR(S): FOUBISTER V; ROSENSHINE I; *FINLAY BB (Reprint)***
CORPORATE SOURCE: UNIV BRITISH COLUMBIA,BIOTECHNOL LAB,ROOM 237,WESTBROOK
BLDG,6174 UNIV BLVD/VANCOUVER V6T 1Z3/BC/CANADA/ (Reprint); UNIV
BRITISH COLUMBIA,BIOTECHNOL LAB/VANCOUVER V6T 1Z3/BC/CANADA/; UNIV
BRITISH COLUMBIA,DEPT BIOCHEM/VANCOUVER V6T 1Z3/BC/CANADA/; UNIV
BRITISH COLUMBIA,DEPT MICROBIOL/VANCOUVER V6T 1Z3/BC/CANADA/
PUBLICATION: JOURNAL OF EXPERIMENTAL MEDICINE, 1994, V179, N3 (MAR 1), P
993-998
ISSN: 0022-1007
LANGUAGE: ENGLISH DOCUMENT TYPE: NOTE

ABSTRACT: Enteropathogenic Escherichia coli (EPEC) is a bacterial pathogen
that causes diarrhea in infants by adhering to intestinal epithelial
cells. EPEC induces host cell *protein*** phosphorylation and increases
intracellular calcium levels that may function to initiate cytoskeletal
rearrangement. We found that EPEC triggers the release of inositol
phosphates (IPs) after adherence of bacteria to cultured epithelial
cells. We also demonstrated that the EPEC-induced flux of IPs precedes
actin rearrangement and bacterial invasion. EPEC mutants and tyrosine
*protein*** kinase inhibitors were used to establish that formation of
IPs is dependent on tyrosine phosphorylation of a *90***-kD*** HeLa
*protein***. Collectively these results suggest that EPEC-induced
tyrosine phosphorylation of a host cell substrate(s) leads to release
of IPs, which may then trigger cytoskeletal rearrangement.

22/3,AB/18 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS
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01088531
METHODS FOR ASSAYING TYPE III SECRETION INHIBITORS
PROCEDES D'ANALYSE D'INHIBITEURS DE SECRETION DE TYPE III
PATENT ASSIGNEE:

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Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3, (CA),
(Applicant designated States: all)

INVENTOR:
FINLAY, Brett, B., Biotechnology Lab. 237-6174 University Boulevard,

Searcher : Shears 308-4994

09/189415

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7HW, (GB)

*STEIN, Marcus***, Via Fiorentina, II, I-53100 Siena, (IT
PATENT (CC, No, Kind, Date):

WO 9945136 990910

APPLICATION (CC, No, Date): EP 99937945 990305; WO 99CA183 990305

PRIORITY (CC, No, Date): US 76980 P 980305

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;
LU; MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: C12Q-001/02; C12Q-001/32; C12Q-001/34;
C12Q-001/42; C12Q-001/48; C12Q-001/66; G01N-033/68

LANGUAGE (Publication, Procedural, Application): English; English; English

22/3,AB/19 (Item 2 from file: 348)

DIALOG(R) File 348:EUROPEAN PATENTS

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01051195

HP90: HOST MEMBRANE RECEPTOR FOR PATHOGENIC BACTERIA, ENCODED BY THE
BACTERIAL TIR GENE

HP90:WIRTSREZEPTOR FÜR PATHOGENE BAKTERIEN

HP90: RECEPTEUR HÔTE À MEMBRANE POUR BACTÉRIES PATHOGENES CODEES PAR LE
GENE BACTERIEN TIR

PATENT ASSIGNEE:

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*DEVINNEY, Rebekah Biotechnology Laboratory***, 237-6174 University
Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)

*STEIN, Marcus IRIS CHIRON S.p.A.***, Via Sioarentina 1, 53100 Sienna, (IT
LEGAL REPRESENTATIVE:

VOSSIUS & PARTNER (100314), Siebertstrasse 4, 81675 Munchen, (DE)

PATENT (CC, No, Kind, Date): EP 1029054 A1 000823 (Basic)

WO 9924576 990520

APPLICATION (CC, No, Date): EP 98954076 981110; WO 98CA1042 981110

PRIORITY (CC, No, Date): US 65130 971112

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;
LU; MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: C12N-015/31; C07K-014/24; C07K-016/12;
G01N-033/53; A61K-038/16; C12Q-001/68; C12N-015/62

NOTE:

Searcher : Shears 308-4994

09/189415

No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/20 (Item 3 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS
(c) 2001 European Patent Office. All rts. reserv.

01003446
PATHOGENIC ESCHERICHIA COLI ASSOCIATED PROTEIN
PATHOGENESE-PROTEIN ESPA VON ESCHERICHIA COLI
PROTEINE ASSOCIEE A UN ESCHERICHIA COLI PATHOGENE
PATENT ASSIGNEE:

THE UNIVERSITY OF BRITISH COLUMBIA, (917327), 222 Health Science Mall,
I.R.C. Building, Vancouver, British Columbia V6T 1Z3, (CA), (applicant
designated states:

AT;BE;CH;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)

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STEIN, Markus, Biotechnology Laboratory", 237-6174 University
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Boulevard, Vancouver, British Columbia V6T 1Z3, (CA)

LEGAL REPRESENTATIVE:

VOSSIUS & PARTNER (100311), Postfach 86 07 67, 81634 Munchen, (DE)

PATENT (CC, No, Kind, Date): EP 904288 A2 990331 (Basic)
WO 9740063 971030

APPLICATION (CC, No, Date): EP 97917185 970423; WO 97CA265

PRIORITY (CC, No, Date): US 15999 P 960423

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU;
MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: C07K-014/00

NOTE:

No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

22/3,AB/21 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0243573 DBA Accession No.: 1999-14338 PATENT
Identifying antibacterial agents that inhibit Gram-negative type-III
secretion system, for treating infections -by screening for inhibition
of virulence factors secreted by this system - e.g. plasmid
pMS21-mediated EspB gene, herpes simplex virus tag gene transfer and
expression in Escherichia coli

Searcher : Shears 308-4994

09/189415

AUTHOR: Finlay B B; *Kenny B***; *Stein M***
CORPORATE SOURCE: Vancouver, British Columbia, Canada.
PATENT ASSIGNEE: Univ.British-Columbia 1999
PATENT NUMBER: WO 9945136 PATENT DATE: 19990910 WPI ACCESSION NO.:
1999-540860 (1945)
PRIORITY APPLIC. NO.: US 76980 APPLIC. DATE: 19980305
NATIONAL APPLIC. NO.: WO 99CA183 APPLIC. DATE: 19990305
LANGUAGE: English

ABSTRACT: Identification of antibacterial agents is new and involves treating bacteria that contain a polynucleotide which encodes a protein secreted by the type-III secretion system (3SS) with a test compound and detecting secretion of the protein. A reduction of secretion, relative to that in bacteria not treated with the test compound, indicates an inhibitor of 3SS. Also claimed is a kit containing in separate containers, the bacteria and a system for detecting secretion of the protein. The antibacterial agents can be used to treat infections in humans other animals and plants, e.g. where caused by enteropathogenic or enterohemorrhagic *Escherichia coli*, *Yershi sp.*, *Shigella sp.*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Xanthomonas campestris* or many others, for analyzing the functional mechanisms of 3SS and for development of more powerful or specific inhibitors. In an example, plasmid pMS21 containing a sequence encoding the N-terminal part of protein EspB and a sequence encoding a herpes simplex virus tag against which commercial antibiotics are available was used to transform 2 enteropathogenic strains of *Escherichia coli* to test for inhibitors. (51pp)

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EspA from enteropathogenic or enterohemorrhagic *Escherichia coli* - vector expression in host cell for recombinant protein production for use as a recombinant vaccine

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PATENT ASSIGNEE: Univ.British-Columbia 1997
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LANGUAGE: English

ABSTRACT: A new secreted EspA protein from *Escherichia coli* with a mol.wt. of 25,000 by SDS-PAGE is encoded by DNA (protein and DNA sequence specified) which can be contained on a vector and used to transform a host cell for production of the recombinant protein. Also claimed is an

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polyclonal or monoclonal antibody which binds to the EspA protein and which can be used to detect EspA in a tissue or biological fluid sample. The presence of EspA indicates infection by enteropathic E. coli. The protein may be used to immunize a host against disease caused by EspA-producing E. coli, or ameliorating such a disease. A DNA probe that hybridizes to the espA nucleic acid molecule can be used to detect espA in a sample. Also claimed is a method of identifying a compound which inhibits bacterial type-II secretion systems, a method for producing a nonpathogenic organism, preferably E. coli, and a method of producing a fusion protein containing EspA and a target protein. (62pp)

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